Towards early recognition of hypogammaglobulinaemia

Janssen, L.M.A.

Publication date:
2022

Document Version
Publisher's PDF, also known as Version of record

TOWARDS EARLY RECOGNITION OF HYPOGAMMAGLOBULINAEMIA

NEW INSIGHTS INTO CLINICAL PRESENTATION PATTERNS AND SCREENING TOOLS

Lisanne Janssen

TOWARDS EARLY RECOGNITION OF HYPOGAMMAGLOBULINAEMIA

NEW INSIGHTS INTO CLINICAL PRESENTATION PATTERNS AND SCREENING TOOLS

Lisanne Janssen

Department of Tranzo
Tilburg University
Tilburg, the Netherlands

Printed by: Ipskamp Printing
Cover design and lay out: ©evelienjagtman.com

ISBN: 978-94-6421-719-3

TOWARDS EARLY RECOGNITION OF HYPOGAMMAGLOBULINAEMIA

NEW INSIGHTS INTO CLINICAL PRESENTATION PATTERNS AND SCREENING TOOLS

Proefschrift
ter verkrijging van de graad van doctor aan Tilburg University,
op gezag van de rector magnificus, prof. dr. W.B.H.J. van de Donk, in het openbaar te
verdedigen ten overstaan van een door het college voor promoties aangewezen
commissie in de Aula van de Universiteit
op maandag 13 juni 2022 om 13.30 uur precies

door

Lisanne Marianne Antoinette Janssen

geboren 7 december 1990
te Duiven.

Promotor:	Prof. dr. E. de Vries (Tilburg University)
Copromotor:	Dr. M. van der Flier (UMC Utrecht)
Leden promotiecommissie:	Prof. dr. A.A. de Bont (Tilburg University) Prof. dr. X. Bossuyt (UZ Leuven) Prof. dr. G.J.A. Driessen (Maastricht UMC) Dr. V.A.S.H. Dalm (Erasmus MC) Dr. C. Obihara (Elisabeth-TweeSteden ziekenhuis)

TABLE OF CONTENTS

Chapter 1

General introduction
and outline of the thesis

GENERAL INTRODUCTION AND OUTLINE OF THE THESIS

Rare diseases are defined as occurring in <5 cases per 10,000 population [1]. Although individually rare, collectively, 1 in 17 persons are affected by a rare disease in their lifetime [2]. Many patients with rare diseases are diagnosed late, particularly those with milder phenotypes presenting at later ages. As a consequence, these patients suffer long uncertainty, multiple hospital attendances, investigations, misdiagnoses, and inappropriate treatments, resulting in huge emotional cost and wasted time, effort and resources [1].

Primary immunodeficiency disorders (PIDs) are rare diseases of the immune system, with more than 400 forms described to date [3]. Predominantly (primary) antibody deficiencies (PADs) are the most prevalent (but still rare) PIDs; they are a typical example of such difficult to recognise rare diseases. PADs lead to higher frequency of infections in the upper and lower airways, often accompanied by severe chronic fatigue [3–6]. PADs can be divided into agammaglobulinaemias, defects of class switch recombination, and hypogammaglobulinaemias. Hypogammaglobulinaemia is by far the most common entity (generally >50%), and in specialised centres, common variable immunodeficiency disorder (CVID) is the most frequent form of hypogammaglobulinaemia seen (estimated prevalence in the population 1:10,000-50,000) [7–9]. Many more patients live with less well described and understood forms of hypogammaglobulinaemia: deficiency of IgG, IgG-subclass(es), IgM, IgA, and/or specific antibodies, alone, or in combination(s) [6]. We refer to these forms as unclassified primary antibody deficiency (unPAD). These generally considered 'milder' hypogammaglobulinaemia patients are often missed [10], because of lack of awareness and incomplete investigations. This thesis focuses on the early detection of hypogammaglobulinaemias, with a specific focus on unPADs.

This introduction discusses the general principles of immunity with a focus on the adaptive immune system, followed by underlying genetic defects and clinical presentation of hypogammaglobulinaemia, with a special emphasis on unPADs, including the problem of diagnostic delay, and explanation why we set up the unPAD study. Finally, the aims of this thesis are outlined.

GENERAL PRINCIPLES OF IMMUNITY

The immune system evolved as a defence against infectious diseases. An immune response consists of five parts: 1) recognition of foreign and dangerous material, 2) an early innate (non-specific) response triggered by this recognition, 3) a slower specific response to a particular antigen (the adaptive response), 4) non-specific augmentation of this response, and 5) memory of specific immune responses, providing a faster and larger response after repeated exposure to that particular antigen. The innate immune response is less efficient, but fast, and involves physical and chemical barriers, circulating effector proteins, and cells with innate phagocytic activity: neutrophils and macrophages. The adaptive immune response is precise, but takes several days or weeks to develop, and consists of antigen-specific reactions through T- and B lymphocyte activities [11]. In contrast to the pattern recognition receptors of the innate immune system, which are of many different types but are not specific for a certain pathogen, the receptors of the adaptive immune system are all of the same molecular type and highly pathogen-specific.

Adaptive immune responses

The adaptive immune system uses antigen-specific receptors on T- and B lymphocytes to drive targeted effector responses in two stages [12]. First, the antigen is presented to and recognised by antigen specific T or B lymphocytes, which leads to cell priming, activation, and differentiation. This usually occurs within the lymphoid tissues. Second, an effector response takes place, either due to activated T lymphocytes leaving the lymphoid tissue and homing to the disease site, or due to the production of antibody from activated B lymphocytes (plasma cells) into blood and tissue fluids, and hence to the focus of infection (**Figure 1.1**). Some of these lymphocytes persist in the body and provide long-term immunological memory. In subsequent encounters with the same pathogen, the memory cells are quickly activated to yield a stronger and faster adaptive immune response, which terminates infections with minimal illness.

The development of B lymphocytes

B lymphocytes develop from pluripotent haematopoietic stem cells in bone marrow and then migrate to secondary lymphoid tissues (i.e. lymph nodes, spleen, Peyer's patches; **Figure 1.2**). During this process a unique B cell antigen receptor (BCR) is created, which is made up of two identical heavy chains (IgH) and two identical light chains (Igk or Igl; each antibody contains either k or l light chains, not both). The immunoglobulin molecule consists of variable (V) and constant (C) regions. The V regions of heavy and light chains together form the antigen-binding site; the variability of this site is responsible for the great diversity of antigen-binding specificities among antibodies. The five main immunoglobulin classes (IgG, IgM, IgD, IgA, IgE) result from differences in the heavy chain C region that result in different physiological properties for each class. The

V regions of immunoglobulin heavy and light chains consist of families of gene segments: variable (V) and joining (J) gene segments in light chains and an additional set of diversity (D) gene segments in heavy chains [13]. During the development of B lymphocytes, the arrays of V, D, and J segments are cut and spliced by DNA recombination. The additional deletion and random insertion of nucleotides at the ends of the V, (D) and J gene segments results in unique junctions, which, in combination with V, (D) and J gene segments contribute enormously to the BCR diversity between precursor-B lymphocytes. First, heavy chains (D_H and J_H) are rearranged in pro-B lymphocytes, followed by joining of a V_H segment to the rearranged DJ_H in the pre-B-I lymphocyte stage [13]. Closest to the rearranged V-region gene is the m C-region gene, which is subsequently transcribed. Once a B lymphocyte expresses a m chain it is known as a pre-B-II lymphocyte, which represents two stages in B lymphocyte development: the less mature large pre-B-II lymphocytes and the more mature small pre-B-II lymphocytes. While rearrangements of heavy chain genes occur in large pre-B-II lymphocytes, rearrangements of light chain genes occur in small pre-B-II lymphocytes. When successful joining of light chain V and J segments is achieved, and light chains are produced with m chains to form membrane-bound IgM, the IgM associates with the Iga and Igb molecules to form a B lymphocyte receptor complex. At this stage the small pre-B-II lymphocytes become immature B lymphocytes. Immature B lymphocytes that are not specific for a self-antigen mature further to express IgM and IgD and leave the bone marrow as transitional B lymphocytes [14]. Mature B lymphocytes that have not yet encountered their antigen are called naïve mature B lymphocytes [15]. Secondary lymphoid tissues provide the sites where naïve mature B lymphocytes can encounter specific antigen. Antigen-specific B lymphocytes become activated by antigen-specific CD4 helper T lymphocytes. After this, some B lymphocytes migrate directly to the medullary cords of the lymph node and differentiate into antibody-secreting plasma cells. Other B lymphocytes migrate into a primary follicle to form a germinal centre. Here, they become large proliferating lymphoblasts (centroblasts), which by inducing somatic hypermutation in their immunoglobulin genes, change their affinity for antigen. The centroblasts mature into more slowly dividing B lymphocytes (centrocytes) and can undergo immunoglobulin class switch recombination [16,17]. Those B lymphocytes that make surface immunoglobulins with the highest affinity for the antigen are selected by the process of affinity maturation and migrate from the germinal centre to other sites in the secondary lymphoid tissue or bone marrow, where they complete their differentiation into plasma cells secreting high-affinity, isotype-switched antibodies [18]. As the primary immune response subsides, germinal centre B lymphocytes also develop into resting memory B lymphocytes possessing high-affinity, isotype-switched antigen receptors.

B lymphocyte responses can also occur independently of T lymphocyte help in the marginal zone of the spleen or in the lamina propria in the gut [19,20]. These B lymphocytes can be activated by the repetitive nature of antigens (i.e. polysaccharide antigens) recognised on blood-borne pathogens (**Figure 1.1**) [21]. The T lymphocyte independent B lymphocyte is called the marginal zone B lymphocyte or natural effector B lymphocyte [22].

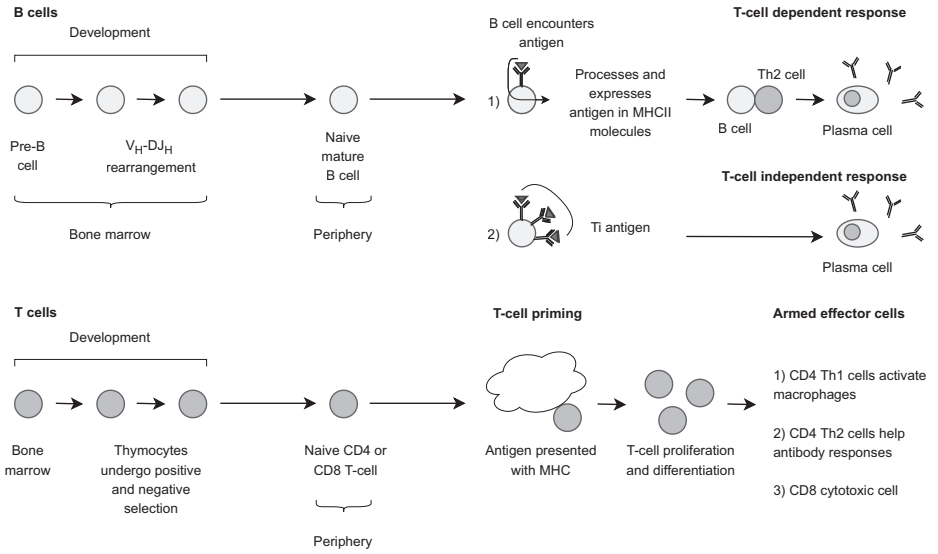


Figure 1.1. The role of T and B lymphocytes in specific immunity.

Figure 1.1. Figure adapted from Parkin et al. [11].

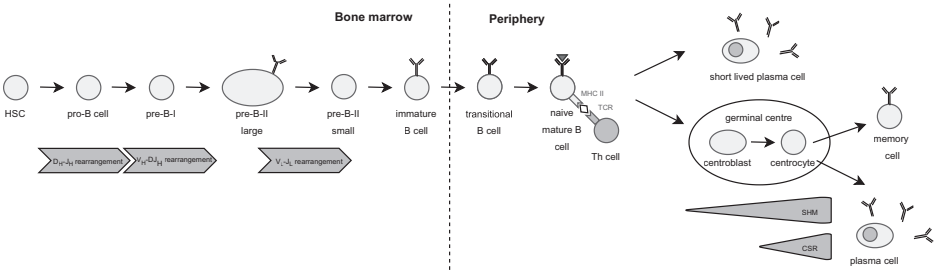


Figure 1.2. B lymphocyte differentiation.

Figure 1.2. Molecular processes during the stepwise differentiation of B lymphocytes from hematopoietic stem cells (HSC) to memory B lymphocytes and plasma cells. Adapted from Thesis Driessen 2013.

PATHOPHYSIOLOGY

The hallmark of hypogammaglobulinaemias is a defect in the production of normal amounts of antibodies with adequate antigen specificity. They result from developmental defects in the B lymphocyte population. While defects in the early stages of B lymphocyte development (mostly defects in the pre-BCR molecule or pre-BCR signalling pathway) can lead to the absence of immunoglobulins (*a*gammaglobulinaemia), B lymphocyte defects in later phases of the developmental pathway can lead to various forms of *hypo*gammaglobulinaemia. The five classes or isotypes of immunoglobulins (IgM, IgD, IgG, IgA and IgE) have different functions in the immune response. IgM is the first antibody produced in an immune response against a pathogen. Surface IgM of the B lymphocyte receptor is monomeric, while secreted effector IgM consists of a circular pentamer of the Y-shaped immunoglobulin monomers. On initiation of an immune response, most of the antibodies that bind the antigen will be of low affinity, and the multiple antigen-binding sites of IgM are needed to enable the antibody to bind sufficiently strong to a pathogen. IgD antibodies are produced in small amounts; their effector function is not known. The most important isotype in blood (IgG) is subdivided into four subclasses (IgG_1, IgG_2, IgG_3, and IgG_4), which are numbered according to their relative abundance in plasma. IgG_1 and IgG_3 subclasses can directly recruit phagocytic cells to ingest antigen-antibody complexes and activate the complement system. As IgG_1 is the most abundant IgG-subclass, IgG_1 deficiency often results in hypogammaglobulinaemia. Of the other IgG-subclass deficiencies, at least IgG_2 deficiency is clinically relevant. Antibodies against encapsulated bacteria are mainly of the IgG_2 subclass, and therefore, the presence of IgG_2 subclass deficiency should alert the clinician to test for concomitant specific anti-polysaccharide antibody deficiency (SPAD) in patients older than two years [23]. IgG_4 is the smallest fraction; IgG_4 deficiency is considered clinically irrelevant [24]. During pregnancy, IgG antibodies are transferred across the placenta. For this reason, unlike patients with T lymphocyte deficiency, patients with antibody deficiency are usually free of infections until 7 to 9 months of age, when maternal antibodies have declined to below protective levels. IgA is the predominant immunoglobulin class in the body [25,26]. Most of the IgA is produced and secreted by plasma cells in the gastrointestinal tract [27,28]. IgA is critical for mucosal immunity. Secreted IgA can be found in tears, milk, saliva, and sweat [29,30]. IgE is specialised in the recruitment of effector functions of mast cells in epithelium, activated eosinophils present at mucosal surfaces, and basophils in blood. When antigen binds the IgE, a strong reaction is triggered that can expel and kill parasites.

Immunoglobulins attach to pathogens (opsonisation) and facilitate their uptake by phagocytes. Therefore, immunoglobulin deficiency leads to recurrent and/or severe infections. It is currently unknown how most forms of milder hypogammaglobulinaemia arise. It seems likely that affected patients form a heterogenous group, where several genetic and environmental factors together determine the clinical phenotype, severity, and outcome.

GENETIC DEFECTS IN PRIMARY ANTIBODY DEFICIENCY

While the genetic basis of most patients with agammaglobulinaemia and class switch recombination (IgCSR) deficiencies has been identified, this is not the case for the majority of patients with hypogammaglobulinaemia. In patients with X-linked agammaglobulinaemia (XLA), the first genetic defect was identified in 1993 in the gene for Bruton's tyrosine kinase (BTK), which is crucial for (pre)B lymphocyte receptor signalling and causes an early block in B lymphocyte development [31,32]. Following the identification of BTK mutations, other genetic defects in components of the preBCR signalling complex were discovered, such as in the Igμ heavy chain [33], l14.1 [34], CD79a [35], BLNK [36] and CD79b [37]. In IgCSR deficiency, marked by disturbed co-stimulation of B and T lymphocytes in the germinal centre, the first genetic defect was identified in the X-linked CD40L gene in 1993 [38], followed the CD40 gene in 2001 [39]. Later, other genetic defects affecting CSR and somatic hypermutation were identified, such as in the AID [40], UNG [41], and PMS2 [42] genes. B lymphocyte defects in later phases of the developmental pathway can lead to various forms of hypogammaglobulinaemia. Only for less than 20% of CVID patients in nonconsanguineous cohorts [9] and approximately 70% of CVID patients in consanguineous cohorts [43], a certain or probable genetic defect has been established, with higher prevalence in complicated CVID patients. This percentage is even lower for patients with milder forms of hypogammaglobulinaemia. This is explained by the highly complex often polygenic genetic basis of hypogammaglobulinaemia and the lack of clarity of many aspects of the disease pathogenesis [44]. The number of identified causal genetic defects is, however, likely to increase over the coming years, because next-generation sequencing is being performed in a growing number of patients. Most genes identified in CVID patients so far are encoding molecules with cellular functions as receptors or signalling components in B lymphocyte development, differentiation, activation, and homeostasis [45,46]. The first identified gene defect in patients with CVID concerned the inducible costimulatory (ICOS) gene in 2003 [47]. In 2005, mutations in TNFRSF13B encoding for TACI (transmembrane activator and CAML interactor) were identified in patients with CVID and IgA deficiency [48,49]. While biallelic TACI mutations are disease-causing, monoallelic mutations only result in increased disease susceptibility, but are not likely to be disease causing because these mutations are also found in healthy individuals [50]. More recent data have revealed a major role for disturbances in the NF-kB pathway [51]. Importantly, the discovery of these genetic aetiologies has revealed that some patients who were originally classified as CVID patients, should actually be classified separately and rather be regarded as combined immunodeficiency patients due to defects in interactions between T and B lymphocytes. This is for example the case in cytotoxic T lymphocyte-associated antigen (CTLA)4 deficiency and LPS-responsive and beige-like anchor protein (LRBA) deficiency [52,53].

SUBGROUPS OF HYPOGAMMAGLOBULINAEMIA

In the European Society for Immunodeficiencies (ESID) online Registry, CVID is strictly defined: age >4 years, markedly decreased serum IgG and IgA with or without IgM, poor antibody response to vaccines, and exclusion of secondary causes (http://www.esid.org). By definition, these patients do not have relevant T lymphocyte deficiency (in laboratory investigations and/or clinically). If they do, they belong to another group (e.g., late-onset combined immunodeficiency (LOCID)) [54]. However, even for CVID, expert opinion varies as to which patients with decreased IgG and disturbed specific antibody responses should be classified under this diagnosis, some considering the combination with decreased IgA or decreased IgM sufficient and others diagnosing CVID only in case IgA is decreased (± decreased IgM) [55]. Subgroups of hypogammaglobulinaemia with isolated immunoglobulin deficiencies have been defined in the ESID Clinical Working Definitions for the ESID online Registry, such as specific antibody deficiency (SPAD), IgA with IgG-subclass deficiency, isolated IgG-subclass deficiency, selective IgM deficiency, and selective IgA deficiency (see **Table 1.1**). All these definitions are solely based upon the results of immunological laboratory investigations. It is not clear how useful they really are. Also, it is important to realise that these definitions can only be used to classify a patient if all relevant laboratory investigations included in the definition have been performed. Because this is often not the case, and because there is currently insufficient evidence that these laboratory-based subgroups have clinical relevance, we prefer to combine all these patients under the umbrella definition of unclassified primary antibody deficiency (unPAD). Within this group, clinical severity as well as the results of immunological laboratory investigations and potential underlying pathophysiology may differ greatly.

Table 1.1. Subgroups based on the ESID Clinical Working Definitions for hypogammaglobulinaemia.

No.	Subgroup	Criteria
1	**Common variable immunodeficiency disorders (CVID)**	Patients with at least one of the following: · Increased susceptibility to infection · Autoimmune manifestations · Granulomatous disease · Unexplained polyclonal lymphoproliferation · Affected family member with antibody deficiency AND marked decrease of IgG and marked decrease of IgA with or without low IgM levels (measured at least twice; <2SD of the normal levels for their age) AND at least one of the following: · Poor antibody response to vaccines (and/or absent Isohemagglutinins) · Low switched memory B lymphocytes (<70% of age-related normal value) AND secondary causes of hypogammaglobulinemia have been excluded AND diagnosis is established after the 4th year of life AND no evidence of profound T-lymphocyte deficiency, defined as 2 out of the following (y=year of life): · CD4 numbers/microliter: 2-6y <300, 6-12y <250, >12y <200 · % naïve CD4: 2-6y <25%, 6-16y <20%, >16 <10% · T lymphocyte proliferation absent
2	**Deficiency of specific IgG (specific antibody deficiency – SPAD)**	Infections (recurrent or severe bacterial) AND normal serum/plasma IgG, A and M and IgG-subclass levels AND profound alteration of the antibody responses to S. pneumonia (or other polysaccharide vaccine) either after documented invasive infection or after test immunization AND exclusion of T-lymphocyte defect
3	**IgA with IgG-subclass deficiency**	Infections (recurrent or severe bacterial) AND undetectable serum/plasma IgA level (with normal/lowish IgG and IgM levels) AND low levels in one of more IgG-subclass (documented twice) AND normal IgG antibody response to some vaccinations AND exclusion of T-lymphocyte defect
4	**Isolated IgG-subclass deficiency**	Infections (recurrent or severe bacterial) AND normal IgG, A and M serum/plasma levels AND low levels in one or more IgG-subclass (documented twice) AND normal IgG antibody response to some vaccinations AND exclusion of T-lymphocyte defect

Table 1.1. Continued.

No.	Subgroup	Criteria
5	**Selective IgM deficiency**	Infections (either invasive or recurrent, usually bacterial) AND low IgM serum/plasma level (with normal IgG and IgG-subclasses and IgA plasma level) AND normal IgG antibody response to all vaccinations AND exclusion of T-lymphocyte defect
6	**Selective IgA deficiency**	At least one of the following: · Increased susceptibility to infection · Autoimmune manifestations · Affected family member AND diagnosis after 4th year of life AND undetectable serum IgA, but normal serum IgG and IgM (measured at least twice) AND secondary causes of hypogammaglobulinemia have been excluded AND normal IgG antibody response to vaccination AND exclusion of T-lymphocyte defect
7	**Unclassified antibody deficiency**	Patients with at least 1 of the following 4: · Recurrent or severe bacterial infections · Autoimmune phenomena (especially cytopenia's) · Polyclonal lymphoproliferation · Affected family member AND at least one of the following: · Marked decrease of at least one of total IgG, IgG1, IgG2, IgG3, IgA or IgM levels · Failure of IgG antibody response(s) to vaccines AND secondary causes of hypogammaglobulinemia have been excluded (infection, protein loss, medication, pregnancy) AND no clinical signs of T-lymphocyte related disease AND does not fit any of the other working definitions (excluding 'unclassified immunodeficiencies')

Source: https://esid.org/Working-Parties/Registry-Working-Party/Diagnosis-criteria (extraction date: 05/03/2021).

CLINICAL MANIFESTATIONS OF HYPOGAMMAGLOBULINAEMIAS

Common variable immunodeficiency (CVID)

Clinical hallmarks of CVID are severe and/or frequent, chronically recurring bacterial infections especially affecting the respiratory tract. As a consequence of these infections, especially in combination with diagnostic delay [56], about one third of patients have already developed bronchiectasis at diagnosis [57]. Chronic *Helicobacter pylori* gastritis and chronic diarrhoea occur frequently; pathogens like *Giardia lamblia, Salmonella,* and *Campylobacter jejuni* are detectable in about half of these cases. Opportunistic infections do not belong to the clinical picture of CVID, but of LOCID [54,58]. Additional non-infectious presentations occur in about one-fifth of patients; they are diverse and include autoimmune diseases, granulomatous diseases, unexplained enteropathy and polyclonal lymphoproliferation [59]. Of the autoimmune diseases, autoimmune thrombocytopenia and autoimmune haemolytic anaemia are particularly common [60]. Less common are autoimmune thyroid disease, vitiligo, pernicious anaemia, psoriasis, rheumatoid arthritis, and systemic lupus erythematosus (SLE) [61,62]. Granulomas can be found in the lungs, lymph nodes, skin, liver, gastrointestinal tract and central nervous system [63]. Gastrointestinal complaints are common in patients with CVID, but often non-specific. A coeliac disease-like presentation is common, but the villous atrophy is generally not gluten-sensitive and is more likely to be due to a distinct autoimmune enteropathy [64]. About 50% of patients have splenomegaly, and about 10-25% lymphadenopathy as signs of benign lymphoproliferation [3,65]. Similar to many PIDs, the incidence of cancer is higher in CVID, especially concerning lymphoid malignancies [66] and gastric carcinomas [67].

Several classification systems have been published that allow the heterogeneous CVID cohort to be grouped into more homogeneous groups that share clinical and immunological characteristics. The "Freiburg" classification, established in 2002, revealed significant clustering of patients with splenomegaly and autoimmune cytopenias in the group of patients with severely reduced class-switched memory $CD27^{+}/IgM^{-}/IgD^{-}$ B lymphocytes (<0.4% of all peripheral blood lymphocytes) and expanded $CD21^{low}$ B lymphocytes (>20%) [68]. In 2003, the "Paris" classification was proposed, showing a higher prevalence of splenomegaly, lymphoid proliferations and granulomatous disease in the patient group with almost no memory B lymphocytes [69]. Hereafter, several follow-up studies have confirmed that classification based on memory B lymphocyte subpopulations is useful for the identification of clinical subtypes of the disease [70–73]. In 2008, the EUROclass classification was published, which confirmed the association of decreased memory B lymphocytes with increased risk of splenomegaly and granulomas and the expansion of $CD21^{low}$ B lymphocytes with splenomegaly, but did not find a clear correlation between $CD21^{low}$ B lymphocytes and autoimmunity [65]. Furthermore, lymphadenopathy was significantly linked with transitional

B lymphocyte expansion. Finally (so far), also in 2008 a clinical classification proposed by Chapel et al. divides patients into five distinct clinical phenotypes comprising patients with no complications, autoimmunity, polyclonal lymphocytic infiltration, enteropathy, and lymphoid malignancy, respectively [59].

Other hypogammaglobulinaemias

Deficiency of IgG, IgG-subclass(es), IgM, IgA, and/or specific antibodies tend to appear in combinations. As single condition they can be asymptomatic, but a combination often leads to more symptoms and sequelae. Other hypogammaglobulinaemic patients also generally present with recurrent "normal" ENT and airway infections caused by common bacterial respiratory agents like pneumococci, *Haemophilus influenzae*, and *Moraxella catarrhalis* [74]. Many suffer from chronic fatigue, leading to a decreased quality of life, increased loss of participation in society (school, work) and higher health care costs [75,76]. When IgA deficiency is also present, gastrointestinal infections with *Giardia lamblia* occur more often. Furthermore, IgA deficiency is associated with atopy and autoimmunity [23,77]. This association is also reported for IgM deficiency, but studies on its clinical significance are difficult to interpret because laboratory investigations are often incomplete and studies have been affected by selection bias towards 'disease', as mostly symptomatic patients from tertiary centre cohorts have been described [78–81]. Anti-polysaccharide antibody deficiency, often occurring with IgG2 deficiency, is associated with an increased susceptibility to encapsulated bacteria [82]. The recurrent infections may lead to irreversible damage to the middle ear, causing hearing loss [83]; the sinuses, causing local obstructive problems [84]; and the lungs, causing bronchiectasis with loss of pulmonary function and a further increased tendency to develop lower respiratory tract infections [85]. Therefore, hypogammaglobulinaemic patients may be "hidden" among patients diagnosed with chronic bronchitis, difficult-to-treat asthma, COPD, unexplained bronchiectasis, or chronic sinusitis. Although rare, patients with hypogammaglobulinaemia may also present with haematological malignancy or autoimmune manifestations. It is important to carefully monitor patients with hypogammaglobulinaemia as the disease may worsen over time and develop into overt CVID, especially when genetic risk factors such as mutations in TNFRSF13B/TACI are present [86].

DIAGNOSTIC CHALLENGE OF HYPOGAMMAGLOBULINAEMIA

Patients with hypogammaglobulinaemia often go unrecognised, because most health care professionals, who are not specialised in immunodeficiency, do not consider potential PID in patients with common symptoms, such as recurrent "normal" infections and chronic fatigue. General practitioners (GPs) see many patients who suffer from such problems, and, in most cases, no rare disease is present, and referral is not necessary. The concomitant fatigue is often interpreted to be of psychosocial origin or labelled as chronic fatigue syndrome. While unPADs are generally not immediately life threatening, a long diagnostic delay can ultimately lead to important morbidity, irreversible organ damage and loss of lifespan when they are not recognised in time and adequately treated. Reducing diagnostic delay is therefore important.

Important screening tests are lymphocyte blood counts, serum immunoglobulin levels (IgM, IgG, and IgA) and evaluation of specific antibody responses to both protein and polysaccharide antigens [10]. Serotype-specific pneumococcal polysaccharide (PnPS) antibody testing is currently accepted as the "gold standard" for the evaluation of anti-polysaccharide antibody production capacity [87]. However, this serotype-specific PnPS testing is not widely available and is time consuming, labour intensive and expensive [88]. The interpretation of the vaccine response is difficult, because uniform reference values are not available and it must be interpreted in the context of the patient's age and immunisation history [89–92]. Therefore, sufficient experience is required to correctly interpret the results. It would be very interesting to search for widely available, reliable and easy-to-interpret screening tests that create a lower screening threshold for antibody deficiency in patients with recurrent infections in secondary care. Ultimately, this will help timely detection of all patients who do have an immunodeficiency.

THE UNPAD STUDY

To reduce the number of missed and unidentified unPADs in the future, the unPAD study was developed. This is a multicentre observational cohort study based on the ESID online Registry data. This study has the intention to describe in detail all types of PAD patients *without* a known specific monogenetic origin regarding their clinical and immunological pattern at presentation and during follow-up, and to identify subgroups based on these clinical and immunological characteristics. Because PADs are rare, international collaboration is necessary to collect sufficient patient numbers for adequate research. A European immunology network (ESID) was formed in 1994, and since 2004 this network has been running an online database for PID patients. In this database, basic characteristics can be registered at first registration and yearly thereafter in the so-called level 1 forms; more detailed characteristics can be registered in level 2 forms, which is mandatory for inclusion in the unPAD study [93]. All patients with 'unclassified antibody deficiency', 'deficiency of specific IgG (specific antibody deficiency – SPAD)', 'IgA with IgG-subclass deficiency', 'isolated IgG-subclass deficiency', 'selective IgM deficiency' and/or 'selective IgA deficiency' [in this project together referred to as 'unPAD patients'] are eligible for analysis in the unPAD study. All data will be monitored and – if necessary – corrected before statistical exploration of the registered data will be performed. Until now, level 1 and 2 forms of 1010 patients have already been monitored, and there is a potential to expand this to about 2000. The unPAD initiative still reaches out to other centres and aims to become a platform that facilitates future collaborative research. Because planning a multicentre study is a lot of work that requires substantial preparation time, and data collection is still ongoing, the results are not presented in this thesis.

AIM AND OUTLINE OF THIS THESIS

The overall aims of the studies described in this thesis are:

1. To improve earlier detection of hypogammaglobulinaemia
2. To increase knowledge of its clinical and immunological presentation patterns

Specific aims of this thesis are:

1. To assess the clinical impact of unPAD
2. To learn more about the clinical significance of truly selective IgM deficiency
3. To better characterise presenting symptoms in children and adults with CVID
4. To explore reasons for PAD patients to seek medical care, and patterns in complaint presentation that led to the diagnosis of PAD
5. To assess the 23-valent anti-PnPS IgG, IgM and IgA screening tests' potential to enable a lower threshold for screening for antibody deficiency

In *chapter 2* we describe a secondary centre cohort with PAD patients, in whom the majority had unPAD. In *chapter 3* we review all previously published patients with decreased serum IgM and describe a cohort of Dutch patients with persistent, isolated decreased serum IgM. In *chapter 4* we describe a larger multicentre European cohort of patients with IgM deficiency using data from the ESID online Registry. In *chapter 5* we review all existing data on the clinical presentation and follow-up of CVID. In *chapter 6* the design and rationale for the unPAD study is presented. *Chapter 7* explores the journey to a PAD diagnosis from the perspective of patients to analyse how these patients appraised their symptoms and which factors were involved in a decision to seek medical care. In *chapter 8* we describe the application of the 23-valent anti-PnPS IgG assay for predicting good responders to pneumococcal polysaccharide vaccination in a general hospital population setting. In *chapter 9* we describe the clinical relevance of 23-valent anti-PnPS IgM and IgA assays in addition to the anti-PnPS IgG assay. The implications of the studies are discussed in the General Discussion (*chapter 10*) which also gives directions for future research.

LITERATURE

1. Schieppati A, Henter J-I, Daina E, Aperia A. Why rare diseases are an important medical and social issue. Lancet (London, England). 2008 Jun;371(9629):2039–41.
2. Evans WR, Rafi I. Rare diseases in general practice: recognising the zebras among the horses. Vol. 66, The British journal of general practice : the journal of the Royal College of General Practitioners. 2016. p. 550–1.
3. Bousfiha AA, Jeddane L, Ailal F, Al Herz W, Conley ME, Cunningham-Rundles C, et al. A phenotypic approach for IUIS PID classification and diagnosis: guidelines for clinicians at the bedside. J Clin Immunol. 2013 Aug;33(6):1078–87.
4. Picard C, Al-Herz W, Bousfiha A, Casanova J-L, Chatila T, Conley ME, et al. Primary Immunodeficiency Diseases: an Update on the Classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015. J Clin Immunol. 2015 Nov;35(8):696–726.
5. de Vries E. Patient-centred screening for primary immunodeficiency, a multi-stage diagnostic protocol designed for non-immunologists: 2011 update. Clin Exp Immunol. 2012 Jan;167(1):108–19.
6. Gathmann B, Grimbacher B, Beaute J, Dudoit Y, Mahlaoui N, Fischer A, et al. The European internet-based patient and research database for primary immunodeficiencies: results 2006-2008. Clin Exp Immunol. 2009 Sep;157 Suppl:3–11.
7. Boyle JM, Buckley RH. Population prevalence of diagnosed primary immunodeficiency diseases in the United States. J Clin Immunol. 2007 Sep;27(5):497–502.
8. Leiva LE, Zelazco M, Oleastro M, Carneiro-Sampaio M, Condino-Neto A, Costa-Carvalho BT, et al. Primary immunodeficiency diseases in Latin America: the second report of the LAGID registry. J Clin Immunol. 2007 Jan;27(1):101–8.
9. Bonilla FA, Barlan I, Chapel H, Costa-Carvalho BT, Cunningham-Rundles C, de la Morena MT, et al. International Consensus Document (ICON): Common Variable Immunodeficiency Disorders. J allergy Clin Immunol Pract. 2016;4(1):38–59.
10. De Vries E. Patient-centred screening for primary immunodeficiency: A multi-stage diagnostic protocol designed for non-immunologists. Clin Exp Immunol. 2006;
11. Parkin J, Cohen B. An overview of the immune system. Lancet (London, England). 2001 Jun;357(9270):1777–89.
12. Bonilla FA, Oettgen HC. Adaptive immunity. J Allergy Clin Immunol. 2010 Feb;125(2 Suppl 2):S33-40.
13. van Zelm MC, van der Burg M, de Ridder D, Barendregt BH, de Haas EFE, Reinders MJT, et al. Ig gene rearrangement steps are initiated in early human precursor B cell subsets and correlate with specific transcription factor expression. J Immunol. 2005 Nov;175(9):5912–22.
14. Sims GP, Ettinger R, Shirota Y, Yarboro CH, Illei GG, Lipsky PE. Identification and characterization of circulating human transitional B cells. Blood. 2005 Jun;105(11):4390–8.
15. Chung JB, Silverman M, Monroe JG. Transitional B cells: step by step towards immune competence. Trends Immunol. 2003 Jun;24(6):343–9.
16. Teng G, Papavasiliou FN. Immunoglobulin somatic hypermutation. Annu Rev Genet. 2007;41:107–20.
17. Stavnezer J, Guikema JEJ, Schrader CE. Mechanism and regulation of class switch recombination. Annu Rev Immunol. 2008;26:261–92.
18. LeBien TW, Tedder TF. B lymphocytes: how they develop and function. Blood. 2008 Sep;112(5):1570–80.
19. Cerutti A. The regulation of IgA class switching. Nat Rev Immunol. 2008 Jun;8(6):421–34.
20. Weill J-C, Weller S, Reynaud C-A. Human marginal zone B cells. Annu Rev Immunol. 2009;27:267–85.

21. Mond JJ, Vos Q, Lees A, Snapper CM. T cell independent antigens. Curr Opin Immunol. 1995 Jun;7(3):349–54.
22. Weller S, Braun MC, Tan BK, Rosenwald A, Cordier C, Conley ME, et al. Human blood IgM "memory" B cells are circulating splenic marginal zone B cells harboring a prediversified immunoglobulin repertoire. Blood. 2004 Dec;104(12):3647–54.
23. Edwards E, Razvi S, Cunningham-Rundles C. IgA deficiency: clinical correlates and responses to pneumococcal vaccine. Clin Immunol. 2004 Apr;111(1):93–7.
24. Ochs HD, Wedgwood RJ. IgG subclass deficiencies. Annu Rev Med. 1987;38:325–40.
25. Conley ME, Delacroix DL. Intravascular and mucosal immunoglobulin A: two separate but related systems of immune defense? Ann Intern Med. 1987 Jun;106(6):892–9.
26. Baştürk B, Sari S, Aral A, Dalgiç B. Prevalence of selective immunoglobulin A deficiency in healthy Turkish school children. Turk J Pediatr. 2011;53(4):364–8.
27. Gommerman JL, Rojas OL, Fritz JH. Re-thinking the functions of IgA(+) plasma cells. Gut Microbes. 2014;5(5):652–62.
28. Brandtzaeg P, Johansen F-E. Mucosal B cells: phenotypic characteristics, transcriptional regulation, and homing properties. Immunol Rev. 2005 Aug;206:32–63.
29. Cohen AB, Goldberg S, London RL. Immunoglobulins in nasal secretions of infants. Clin Exp Immunol. 1970 May;6(5):753–60.
30. McKay E, Thom H. Observations on neonatal tears. J Pediatr. 1969 Dec;75(6):1245–56.
31. BRUTON OC. Agammaglobulinemia. Pediatrics. 1952 Jun;9(6):722–8.
32. Vetrie D, Vorechovský I, Sideras P, Holland J, Davies A, Flinter F, et al. The gene involved in X-linked agammaglobulinaemia is a member of the src family of protein-tyrosine kinases. Nature. 1993 Jan;361(6409):226–33.
33. Yel L, Minegishi Y, Coustan-Smith E, Buckley RH, Trübel H, Pachman LM, et al. Mutations in the mu heavy-chain gene in patients with agammaglobulinemia. N Engl J Med. 1996 Nov;335(20):1486–93.
34. Minegishi Y, Coustan-Smith E, Wang YH, Cooper MD, Campana D, Conley ME. Mutations in the human lambda5/14.1 gene result in B cell deficiency and agammaglobulinemia. J Exp Med. 1998 Jan;187(1):71–7.
35. Minegishi Y, Coustan-Smith E, Rapalus L, Ersoy F, Campana D, Conley ME. Mutations in Igalpha (CD79a) result in a complete block in B-cell development. J Clin Invest. 1999 Oct;104(8):1115–21.
36. Minegishi Y, Rohrer J, Coustan-Smith E, Lederman HM, Pappu R, Campana D, et al. An essential role for BLNK in human B cell development. Science. 1999 Dec;286(5446):1954–7.
37. Dobbs AK, Yang T, Farmer D, Kager L, Parolini O, Conley ME. Cutting edge: a hypomorphic mutation in Igbeta (CD79b) in a patient with immunodeficiency and a leaky defect in B cell development. J Immunol. 2007 Aug;179(4):2055–9.
38. Aruffo A, Farrington M, Hollenbaugh D, Li X, Milatovich A, Nonoyama S, et al. The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome. Cell. 1993 Jan;72(2):291–300.
39. Ferrari S, Giliani S, Insalaco A, Al-Ghonaium A, Soresina AR, Loubser M, et al. Mutations of CD40 gene cause an autosomal recessive form of immunodeficiency with hyper IgM. Proc Natl Acad Sci U S A. 2001 Oct;98(22):12614–9.
40. Revy P, Muto T, Levy Y, Geissmann F, Plebani A, Sanal O, et al. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). Cell. 2000 Sep;102(5):565–75.

41. Imai K, Slupphaug G, Lee W-I, Revy P, Nonoyama S, Catalan N, et al. Human uracil-DNA glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch recombination. Nat Immunol. 2003 Oct;4(10):1023–8.
42. Péron S, Metin A, Gardès P, Alyanakian M-A, Sheridan E, Kratz CP, et al. Human PMS2 deficiency is associated with impaired immunoglobulin class switch recombination. J Exp Med. 2008 Oct;205(11):2465–72.
43. Abolhassani H, Aghamohammadi A, Fang M, Rezaei N, Jiang C, Liu X, et al. Clinical implications of systematic phenotyping and exome sequencing in patients with primary antibody deficiency. Genet Med. 2019 Jan;21(1):243–51.
44. Ameratunga R, Lehnert K, Woon S-T, Gillis D, Bryant VL, Slade CA, et al. Review: Diagnosing Common Variable Immunodeficiency Disorder in the Era of Genome Sequencing. Clin Rev Allergy Immunol. 2018 Apr;54(2):261–8.
45. Yong PFK, Thaventhiran JED, Grimbacher B. "A rose is a rose is a rose," but CVID is Not CVID common variable immune deficiency (CVID), what do we know in 2011? Adv Immunol. 2011;111:47–107.
46. Maffucci P, Filion CA, Boisson B, Itan Y, Shang L, Casanova J-L, et al. Genetic Diagnosis Using Whole Exome Sequencing in Common Variable Immunodeficiency. Front Immunol. 2016;7:220.
47. Grimbacher B, Hutloff A, Schlesier M, Glocker E, Warnatz K, Dräger R, et al. Homozygous loss of ICOS is associated with adult-onset common variable immunodeficiency. Nat Immunol. 2003 Mar;4(3):261–8.
48. Salzer U, Chapel HM, Webster ADB, Pan-Hammarström Q, Schmitt-Graeff A, Schlesier M, et al. Mutations in TNFRSF13B encoding TACI are associated with common variable immunodeficiency in humans. Nat Genet. 2005 Aug;37(8):820–8.
49. Castigli E, Wilson SA, Garibyan L, Rachid R, Bonilla F, Schneider L, et al. TACI is mutant in common variable immunodeficiency and IgA deficiency. Nat Genet. 2005 Aug;37(8):829–34.
50. Zhang L, Radigan L, Salzer U, Behrens TW, Grimbacher B, Diaz G, et al. Transmembrane activator and calcium-modulating cyclophilin ligand interactor mutations in common variable immunodeficiency: clinical and immunologic outcomes in heterozygotes. J Allergy Clin Immunol. 2007 Nov;120(5):1178–85.
51. Keller B, Cseresnyes Z, Stumpf I, Wehr C, Fliegauf M, Bulashevska A, et al. Disturbed canonical nuclear factor of κ light chain signaling in B cells of patients with common variable immunodeficiency. J Allergy Clin Immunol. 2017 Jan;139(1):220-231.e8.
52. Schubert D, Bode C, Kenefeck R, Hou TZ, Wing JB, Kennedy A, et al. Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. Nat Med. 2014 Dec;20(12):1410–6.
53. Lopez-Herrera G, Tampella G, Pan-Hammarström Q, Herholz P, Trujillo-Vargas CM, Phadwal K, et al. Deleterious mutations in LRBA are associated with a syndrome of immune deficiency and autoimmunity. Am J Hum Genet. 2012 Jun;90(6):986–1001.
54. Malphettes M, Gerard L, Carmagnat M, Mouillot G, Vince N, Boutboul D, et al. Late-onset combined immune deficiency: a subset of common variable immunodeficiency with severe T cell defect. Clin Infect Dis. 2009 Nov;49(9):1329–38.
55. Ameratunga R, Woon S-T. Perspective: Evolving Concepts in the Diagnosis and Understanding of Common Variable Immunodeficiency Disorders (CVID). Clin Rev Allergy Immunol. 2019 Nov;
56. Odnoletkova I, Kindle G, Quinti I, Grimbacher B, Knerr V, Gathmann B, et al. The burden of common variable immunodeficiency disorders: a retrospective analysis of the European Society for Immunodeficiency (ESID) registry data. Orphanet J Rare Dis. 2018 Nov;13(1):201.

57. Gathmann B, Mahlaoui N, Gerard L, Oksenhendler E, Warnatz K, Schulze I, et al. Clinical picture and treatment of 2212 patients with common variable immunodeficiency. J Allergy Clin Immunol. 2014 Jul;134(1):116–26.
58. Bertinchamp R, Gérard L, Boutboul D, Malphettes M, Fieschi C, Oksenhendler E. Exclusion of Patients with a Severe T-Cell Defect Improves the Definition of Common Variable Immunodeficiency. J allergy Clin Immunol Pract. 2016;4(6):1147–57.
59. Chapel H, Lucas M, Lee M, Bjorkander J, Webster D, Grimbacher B, et al. Common variable immunodeficiency disorders: division into distinct clinical phenotypes. Blood. 2008 Jul;112(2):277–86.
60. Feuille EJ, Anooshiravani N, Sullivan KE, Fuleihan RL, Cunningham-Rundles C. Autoimmune Cytopenias and Associated Conditions in CVID: a Report From the USIDNET Registry. J Clin Immunol. 2018 Jan;38(1):28–34.
61. Boileau J, Mouillot G, Gerard L, Carmagnat M, Rabian C, Oksenhendler E, et al. Autoimmunity in common variable immunodeficiency: correlation with lymphocyte phenotype in the French DEFI study. J Autoimmun. 2011 Feb;36(1):25–32.
62. Fischer A, Provot J, Jais J-P, Alcais A, Mahlaoui N. Autoimmune and inflammatory manifestations occur frequently in patients with primary immunodeficiencies. J Allergy Clin Immunol. 2017 Nov;140(5):1388-1393.e8.
63. Ardeniz O, Cunningham-Rundles C. Granulomatous disease in common variable immunodeficiency. Clin Immunol. 2009 Nov;133(2):198–207.
64. Jorgensen SF, Reims HM, Frydenlund D, Holm K, Paulsen V, Michelsen AE, et al. A Cross-Sectional Study of the Prevalence of Gastrointestinal Symptoms and Pathology in Patients With Common Variable Immunodeficiency. Am J Gastroenterol. 2016 Oct;111(10):1467–75.
65. Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, et al. The EUROclass trial: defining subgroups in common variable immunodeficiency. Blood. 2008 Jan;111(1):77–85.
66. Chua I, Quinti I, Grimbacher B. Lymphoma in common variable immunodeficiency: interplay between immune dysregulation, infection and genetics. Curr Opin Hematol. 2008 Jul;15(4):368–74.
67. Pulvirenti F, Pecoraro A, Cinetto F, Milito C, Valente M, Santangeli E, et al. Gastric Cancer Is the Leading Cause of Death in Italian Adult Patients With Common Variable Immunodeficiency. Front Immunol. 2018;9:2546.
68. Warnatz K, Wehr C, Drager R, Schmidt S, Eibel H, Schlesier M, et al. Expansion of CD19(hi)CD21(lo/neg) B cells in common variable immunodeficiency (CVID) patients with autoimmune cytopenia. Immunobiology. 2002 Dec;206(5):502–13.
69. Piqueras B, Lavenu-Bombled C, Galicier L, Bergeron-van der Cruyssen F, Mouthon L, Chevret S, et al. Common variable immunodeficiency patient classification based on impaired B cell memory differentiation correlates with clinical aspects. J Clin Immunol. 2003 Sep;23(5):385–400.
70. Sánchez-Ramón S, Radigan L, Yu JE, Bard S, Cunningham-Rundles C. Memory B cells in common variable immunodeficiency: clinical associations and sex differences. Clin Immunol. 2008 Sep;128(3):314–21.
71. Alachkar H, Taubenheim N, Haeney MR, Durandy A, Arkwright PD. Memory switched B cell percentage and not serum immunoglobulin concentration is associated with clinical complications in children and adults with specific antibody deficiency and common variable immunodeficiency. Clin Immunol. 2006 Sep;120(3):310–8.
72. Detková D, de Gracia J, Lopes-da-Silva S, Vendrell M, Alvarez A, Guarner L, et al. Common variable immunodeficiency: association between memory B cells and lung diseases. Chest. 2007 Jun;131(6):1883–9.

73. Ko J, Radigan L, Cunningham-Rundles C. Immune competence and switched memory B cells in common variable immunodeficiency. Clin Immunol. 2005 Jul;116(1):37–41.
74. Ochs HD, Stiehm ER, Winkelstein JA et al. Immunologic disorders in infants and children. 5th ed. Philadelphia; 2004.
75. Aghamohammadi A, Montazeri A, Abolhassani H, Saroukhani S, Pourjabbar S, Tavassoli M, et al. Health-related quality of life in primary antibody deficiency. Iran J Allergy Asthma Immunol. 2011 Mar;10(1):47–51.
76. Barton JC, Bertoli LF, Barton JC, Acton RT. Fibromyalgia in 300 adult index patients with primary immunodeficiency. Clin Exp Rheumatol. 2017;35 Suppl 1(3):68–73.
77. Jacob CMA, Pastorino AC, Fahl K, Carneiro-Sampaio M, Monteiro RC. Autoimmunity in IgA deficiency: revisiting the role of IgA as a silent housekeeper. J Clin Immunol. 2008 May;28 Suppl 1:S56-61.
78. Yel L, Ramanuja S, Gupta S. Clinical and immunological features in IgM deficiency. Int Arch Allergy Immunol. 2009;150(3):291–8.
79. Goldstein MF, Goldstein AL, Dunsky EH, Dvorin DJ, Belecanech GA, Shamir K. Selective IgM immunodeficiency: retrospective analysis of 36 adult patients with review of the literature. Ann Allergy Asthma Immunol. 2006 Dec;97(6):717–30.
80. Goldstein MF, Goldstein AL, Dunsky EH, Dvorin DJ, Belecanech GA, Shamir K. Pediatric selective IgM immunodeficiency. Clin Dev Immunol. 2008;2008:624850.
81. Entezari N, Adab Z, Zeydi M, Saghafi S, Jamali M, Kardar GA, et al. The prevalence of Selective Immunoglobulin M Deficiency (SIgMD) in Iranian volunteer blood donors. Hum Immunol. 2016 Jan;77(1):7–11.
82. Cheng YK, Decker PA, O'Byrne MM, Weiler CR. Clinical and laboratory characteristics of 75 patients with specific polysaccharide antibody deficiency syndrome. Ann allergy, asthma Immunol Off Publ Am Coll Allergy, Asthma, Immunol. 2006 Sep;97(3):306–11.
83. Tavakol M, Kouhi A, Abolhassani H, Ghajar A, Afarideh M, Shahinpour S, et al. Otological findings in pediatric patients with hypogammaglobulinemia. Iran J Allergy Asthma Immunol. 2014 Jun;13(3):166–73.
84. Chiarella SE, Grammer LC. Immune deficiency in chronic rhinosinusitis: screening and treatment. Expert Rev Clin Immunol. 2017 Feb;13(2):117–23.
85. Ruffner MA, Aksamit TR, Thomashow B, Choate R, DiMango A, Turino GM, et al. Frequency of untreated hypogammaglobulinemia in bronchiectasis. Ann allergy, asthma Immunol Off Publ Am Coll Allergy, Asthma, Immunol. 2017 Jul;119(1):83–5.
86. Rachid R, Castigli E, Geha RS, Bonilla FA. TACI mutation in common variable immunodeficiency and IgA deficiency. Curr Allergy Asthma Rep. 2006 Sep;6(5):357–62.
87. Orange JS, Ballow M, Stiehm ER, Ballas ZK, Chinen J, De La Morena M, et al. Use and interpretation of diagnostic vaccination in primary immunodeficiency: a working group report of the Basic and Clinical Immunology Interest Section of the American Academy of Allergy, Asthma & Immunology. J Allergy Clin Immunol. 2012 Sep;130(3 Suppl):S1-24.
88. Daly TM, Hill HR. Use and clinical interpretation of pneumococcal antibody measurements in the evaluation of humoral immune function. Clin Vaccine Immunol. 2015 Feb;22(2):148–52.
89. Borgers H, Moens L, Picard C, Jeurissen A, Raes M, Sauer K, et al. Laboratory diagnosis of specific antibody deficiency to pneumococcal capsular polysaccharide antigens by multiplexed bead assay. Clin Immunol. 2010 Feb;134(2):198–205.

90. Balloch A, Licciardi P V, Tang MLK. Serotype-specific anti-pneumococcal IgG and immune competence: critical differences in interpretation criteria when different methods are used. J Clin Immunol. 2013 Feb;33(2):335–41.
91. Hare ND, Smith BJ, Ballas ZK. Antibody response to pneumococcal vaccination as a function of preimmunization titer. J Allergy Clin Immunol. 2009 Jan;123(1):195–200.
92. Balmer P, Cant AJ, Borrow R. Anti-pneumococcal antibody titre measurement: what useful information does it yield? J Clin Pathol. 2007 Apr;60(4):345–50.
93. Guzman D, Veit D, Knerr V, Kindle G, Gathmann B, Eades-Perner AM, et al. The ESID Online Database network. Bioinformatics. 2007 Mar;23(5):654–5.

PART I

Describing hypogammaglobulinaemia

Chapter 2

Mild Hypogammaglobulinemia can be a Serious Condition

Lisanne MA Janssen, MD, Paul Bassett, Msc, Thomas Macken, MD, Jolanda van Esch, Hans Pruijt, MD, Arnoud Knoops, MD, PhD, Markus Sköld, PhD, Antony Parker, PhD, Jolanda de Vries, PhD, Esther de Vries, MD, PhD

Frontiers in Immunology. 2018; 9:2384

ABSTRACT

Background

Most patients with primary antibody deficiency (PAD) suffer from less-well described and understood forms of hypogammaglobulinemia (unclassified primary antibody deficiency, unPAD). Because of the moderately decreased immunoglobulin levels compared to CVID, unPAD is generally considered to be clinically mild and not very relevant.

Objective

To describe our cohort of - mainly - unPAD patients, and to analyse whether subgroups can be identified.

Methods

Data were prospectively collected (February-2012 to June-2016) as part of a standardized, 1-day Care Pathway for suspected primary immunodeficiency. The TNO-AZL Questionnaire for Health-Related Quality of Life (HRQoL) was part of the pre-first-visit intake procedure.

Results

320 patients were referred to the Care Pathway. Data from 23/27 children and 99/113 adults who were diagnosed with PAD and gave informed consent were available for analysis. 89/99 adults had unPAD, the majority (74%) were female and 44% already showed bronchiectasis. HRQoL was significantly decreased in *all* domains, meaning that a lot of unPAD patients had to cope simultaneously with pain, negative feelings and impairments in cognition, home management tasks, sleep, social interaction, and work. The most prominently impaired HRQoL domain was vitality, indicating these patients feel extremely tired and worn out.

Conclusion

These results highlight the need for more attention to the potential patient burden of unPADs. A larger cohort is needed to increase our understanding of unPADs and to analyse whether distinct subgroups can be identified. For now, it is important for the clinician to acknowledge the existence of unPAD and be aware of its potential consequences, in order to timely and appropriately manage its effects and complications.

INTRODUCTION

Primary immune deficiencies (PIDs) are rare, inherited defects of the immune system with more than 300 forms described to date [1]. Only a small subgroup of patients suffer from a form of PID that leads to significant problems very early in life [2]. Most PID patients have PID forms that are less severe and present later in life [1–4], mainly comprising of diseases with predominantly (primary) antibody deficiency (PAD). PADs can be divided into agammaglobulinemias, defects of class switch recombination, and hypogammaglobulinemias. Hypogammaglobulinemia is by far the most common entity, comprising nearly half of all PID diagnoses [2,4]. In specialized centres, common variable immunodeficiency disorder (CVID) is the most common form of hypogammaglobulinemia seen (estimated prevalence in the population 1:10,000-50,000) [5,6]. In the ESID Registry, CVID is strictly defined: age >4 years, markedly decreased serum IgG and IgA with or without low IgM, poor antibody response to vaccines, and exclusion of an underlying cause (http://www.esid.org). Many more patients live with less well described and understood forms of hypogammaglobulinemia: deficiency of IgG, IgG-subclass(es), IgM, IgA and/or specific antibodies, alone, or in combination(s) [4]. We refer to these forms as *unclassified* primary antibody deficiency (unPAD). Because of the moderately decreased immunoglobulin levels, unPADs are generally considered to be clinically milder. However, data regarding the clinical presentation, prognosis and treatment of unPAD patients are limited. These patients generally do not visit physicians in specialized/tertiary centres, and are often not treated with the immunodeficiency taken into consideration.

The symptoms of patients with hypogammaglobulinemia can lead to decreased quality of life, increased loss of participation in society (school, work) and higher health care costs [7–12]. These patients often go unrecognized, because the general public as well as most health care professionals, who are not specialized in immunodeficiency, do not consider potential PID in patients with recurrent 'normal' infections (responding to regular treatment, and not caused by an unexpected or opportunistic pathogen). Their concomitant fatigue is often interpreted to be of psychosocial origin or labelled as chronic fatigue syndrome.

To improve awareness and early detection of PID in patients with recurrent normal infections, we developed a Care Pathway for suspected primary immunodeficiency in the Jeroen Bosch hospital (JBZ), a large teaching hospital (secondary centre) in the southern part of the Netherlands. All patient data obtained in regular care in the Pathway were collected and stored electronically. In this report, we present a detailed analysis of the available clinical data from the patients diagnosed with PAD during the first four years of the Pathway who gave informed consent for using their data for this purpose; these were mostly unPAD patients.

MATERIAL AND METHODS

Study design

Data were prospectively collected, pseudonymized, and stored on a protected server using Research Manager software developed by Cloud9 Health Solutions (Deventer, the Netherlands) as part of a standardized, 1-day Care Pathway for suspected PID. Data were collected on all patients referred to the Care Pathway from the start in February 2012 to June 2016. The primary objective of this study was to describe the patients, with special focus on unPAD patients, for this project including 'deficiency of specific IgG (specific antibody deficiency – SPAD)', 'IgA with IgG subclass deficiency', 'isolated IgG subclass deficiency', 'selective IgM deficiency' and 'selective IgA deficiency'. The secondary objective was to analyse whether subgroups could be identified. Only participants who gave written informed consent were included in this study; the Medical Ethical Committee Brabant approved the study.

The Care Pathway

Patients were referred to the Care Pathway when there was a suspicion of potential immunodeficiency. Upon referral, data were collected electronically on patient and family history, and previous medical information was requested. Based on this information, the immunologist (author De Vries) decided whether visiting the Care Pathway was indicated, based on the clinical presentations of PID as presented in the ESID diagnostic protocol [3,13]. Patients could be referred by general practitioners or by medical specialists (e.g. pulmonologists, internists, ENT-surgeons, dermatologists). 77% lived in the encachment area of the JBZ (320,000 people), the remaining patients were referred from other parts of the country.

The Care Pathway comprised a visit to an immunologist specialized in the field of PID (author De Vries), in addition to indicated laboratory and radiologic evaluations, and pulmonary function tests. After completion of the Care Pathway, each patient was evaluated in a multidisciplinary team, attended by the immunologist, a pulmonologist, an internist, and specialized nurse. The team formulated an advice for each individual patient on: 1) presence or absence of PID, 2) indication for treatment with immunoglobulin substitution and/or (change of) antimicrobial prophylaxis, 3) indication for investigation of family members, and 4) necessity of referral to a tertiary centre.

Assessments

All assessments were performed during regular, routine patient care and included online questionnaires to be completed by patients, laboratory tests, pulmonary function tests and imaging. All patients recorded the following social, demographic and clinical characteristics: age, gender, smoking habit, previous symptoms, prescribed therapy, family history, and highest education level. Health-related quality of life (HRQoL) was measured using the age specific

TNO-AZL questionnaires: TNO-AZL Pre-school children's Quality of Life questionnaire (TAPQOL, *parents* for children aged 1-5 years), TNO-AZL Children's Quality of Life questionnaire (TACQOL, *parents* and *children* for children aged 6-15 and 8-15 years, respectively), or TNO-AZL questionnaire for adult's HRQoL (TAAQOL, ≥16 years) [14]. Items inquire about the incidence of physical, psychological or social problems on different domains and are scored on a 3- or 4-point Likert scale (TAPQOL & TACQOL 'never / occasionally / often' a problem with …; TAAQOL 'no / a little / some / a lot of' difficulty in …). If a problem/difficulty is reported, parents and children 8-15 years rate how the children felt at those times on a 4-point Likert scale (TAPQOL 'well / not very well / unwell / very unwell'; TACQOL 'fine / not so good / quite bad / bad'); children 16 years and over and adult subjects rate how much that problem bothered them on a 4-point Likert scale (TAAQOL 'not at all / a little / quite a lot / very much'). Higher scores indicate a better HRQoL. For interpretation of the various laboratory tests, age-matched reference values were used. For interpretation of pneumococcal antibody responses laboratory specific reference values were used[1]. Analysis of B- and T-cell subpopulations were performed as described previously [15].The High-resolution CT (HRCT) scans of the thorax were scored by a thoracic radiologist according to the 'Chest CT in ADS' criteria[2]. Finally, the immunologist scored the diagnosis, first clinical presentation [3,13] and disease status of the patient.

Statistical analysis

Continuous variables were summarized by mean and standard deviation (SD) when normally distributed, and otherwise by median and inter-quartile range (IQR). Categorical variables were summarized by number and percentage. The group of children with PAD was too small to perform statistical analyses.

The domain scores of the TAPQOL, TACQOL and TAAQOL were computed using the SPSS syntax provided by the authors. To ensure similarity with the Care Pathway data, individuals outside the observed age range of the Care Pathway patients (21–77) were excluded from the adult reference data before analysis, leaving data from 4,120 subjects in the reference dataset. Analyses examining the associations between categorical variables in the adult patients were performed using Fisher's exact test. Analyses examining the differences in continuous measures in the adult patients were performed using the unpaired t-test when normally distributed, and otherwise by the Mann-Whitney test. Because of the great number of comparisons, only p-values < 0.01 were regarded to be statistically significant. The spearman's rank correlation coefficient test was used to examine the association between IgG subclass levels and pneumococcal vaccination response. The following cut-off values were used to describe r: 0.00 to 0.19 "very weak"; 0.20 to 0.39 "weak"; 0.40 to 0.59 "moderate"; 0.60 to 0.79 "strong"; and 0.80 to 1.0 "very strong".

1 www.umcutrecht.nl/subsite/medische-immunologie/diagnostiek.

2 www.chest-ct-group.eu.

To analyse whether the adult patient group could be divided into subgroups, K-means clustering was applied creating between 2 to 5 clusters. Variables that exhibited highly positively skewed distributions were analysed on the log scale. The Calinski/Harabasz pseudo-F index was calculated for each cluster solution; the solution with the largest value of this index indicated the most distinct clustering, and was chosen as the optimal solution. Next, the differences between clusters were examined, by comparing variables between the clusters.

The logistic regression model was used for evaluating the predictive effect of family history, TAAQOL domains and HRCT findings for the patient diagnosis, classified as either CVID (according to the ESID Registry working diagnosis criteria) [16] or unPAD. For factors where the diagnosis was the same for all patients in a category, it was not mathematically possible to perform logistic regression, and Fisher's exact test was used instead. Firstly, the association between each factor and the outcome was examined separately in a series of univariable analyses. Subsequently, the joint association of the factors and the outcome was examined in a multivariable analysis. To restrict the number of variables in the second stage of the analysis, only those factors with a univariable p-value of ≤ 0.10 were used for this stage. A backwards selection procedure was used to retain only the statistically significant variables in the final model. This involves omitting non-significant variables, one at a time, until only the significant variables remain.

RESULTS

The Care Pathway

From the 320 patients that were referred to the Care Pathway between February 2012 and June 2016, 153 were shown to have some form of PID (92% PAD). In 99 adults and 23 children with PAD, written informed consent for inclusion in this analysis was obtained (details of patient selection process in **Figure 2.1**).

Description of the paediatric patients

The group of 23 children with PAD was too small to perform any meaningful statistical analysis. An overview of their collected data is shown in **Supplementary Table 2.1**. The parents' main complaint at referral was that their child was 'ill too often' in 70%, for 83% the main reason for referral was to find out the reason why ('what is the matter with him/her'). 15 children were referred by their paediatrician, 4 by their general practitioner, 3 by their ENT surgeon, and 1 by their dermatologist. Age at referral was 2-16 yrs (mean 7.5 yrs; median 6 yrs); boys predominated (74%). The clinical presentation [3,13] was 'recurrent ENT and airway infections' in 78%. 39% had an iron deficiency and 39% had an increased total IgE, one third of these had ≥1 positive specific IgE in their serum (tree pollen, house dust mite, cat dander, dog dander and/or grass pollen). In 22%, other family member(s) also had a PAD diagnosis (already known in three, not yet known in two cases). A pulmonary HRCT scan was performed in five patients; two showed bronchopathy, none bronchiectasis. In 41%, a mild to moderate decrease in HRQoL was reported. Five were put on prophylactic co-trimoxazole, two on subcutaneous and two on intravenous immunoglobulin substitution.

Description of the adult patients

Of the 99 PAD patients (71 women, 72%), 89 had unPAD (66 women, 74%), and 10 had CVID (5 women, 50%) according to the working diagnoses used in the ESID online Registry [16]. An overview of their collected data is shown in **Supplementary Table 2.2**. Age at referral was 21.0-77.4 yrs (mean 51.3 yrs; median 51.5 yrs). BMI, smoking habits, and highest educational level were comparable with the Dutch LISS panel, a representative sample of the Dutch population (http://www.lissdata.nl). The majority (n=43) was referred by their pulmonologist, followed by their general practitioner (n=35), and internist (n=15). The wide variety of clinical specialists the patients with PAD had visited prior to their referral to the Care Pathway is illustrated in **Figure 2.2**. The main complaint at referral was 'ill too often' in 87, with airway infections in 35, chronic cough in 4, and ENT-infections in 9 patients. For 35 patients being extremely tired and having no energy was their most important complaint. The immunologist characterized the initial presentation as 'recurrent ENT and airway infections' in 88, 'auto-immune or chronic inflammatory disease; lymphoproliferation' in 10, and 'unusual infections or unusually severe course of infections' in 1 of the patients [13].

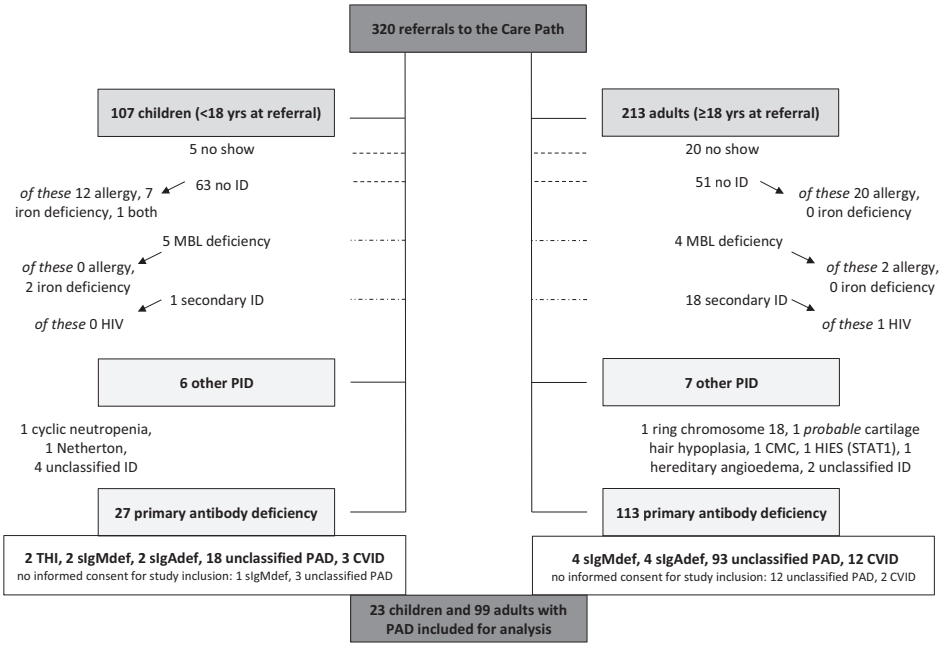


Figure 2.1. The population under study.

Figure 2.1. Overview of all patients referred to the Care Pathway between February 2012 and June 2016 (inclusive) to answer the question 'could this be primary immunodeficiency?'. When taking all details of the ESID Registry Working Diagnoses into account, sIgAdef, sIgMdef and unclassified PAD in these patients all fall under the Working Diagnosis 'unclassified antibody deficiency' (unPAD) [16].

Abbreviations: allergy = clinical symptoms and proven sensitization by specific serum IgE and/or skin prick test; CVID = common variable immunodeficiency disorders (according to the ESID Registry working diagnoses); HIV = human immunodeficiency virus; Ig = immunoglobulin; iron deficiency = low iron stores, determined by serum ferritin level; MBL = mannose binding lectin; (other) (P)ID = (other) (primary) immunodeficiency (other meaning other than primary antibody deficiency); sIgAdef = selective IgA deficiency (according to the ESID Registry working diagnoses, but absence of clinical signs of T-cell deficiency was considered sufficient); sIgMdef = selective IgM deficiency (according to the ESID Registry working diagnoses, but absence of clinical signs of T-cell deficiency was considered sufficient); THI = definite transient hypogammaglobulinemia of infancy (the antibody deficiency has resolved during the period under study); unclassified ID resp. PAD = unclassified primary antibody resp. immuno-deficiency (according to the ESID Registry working diagnoses); yrs = years.

As expected by the clinical definition of CVID and unPAD, the median immunoglobulin levels at diagnosis were lower in patients with CVID compared to unPAD patients (**Figure 2.3**).

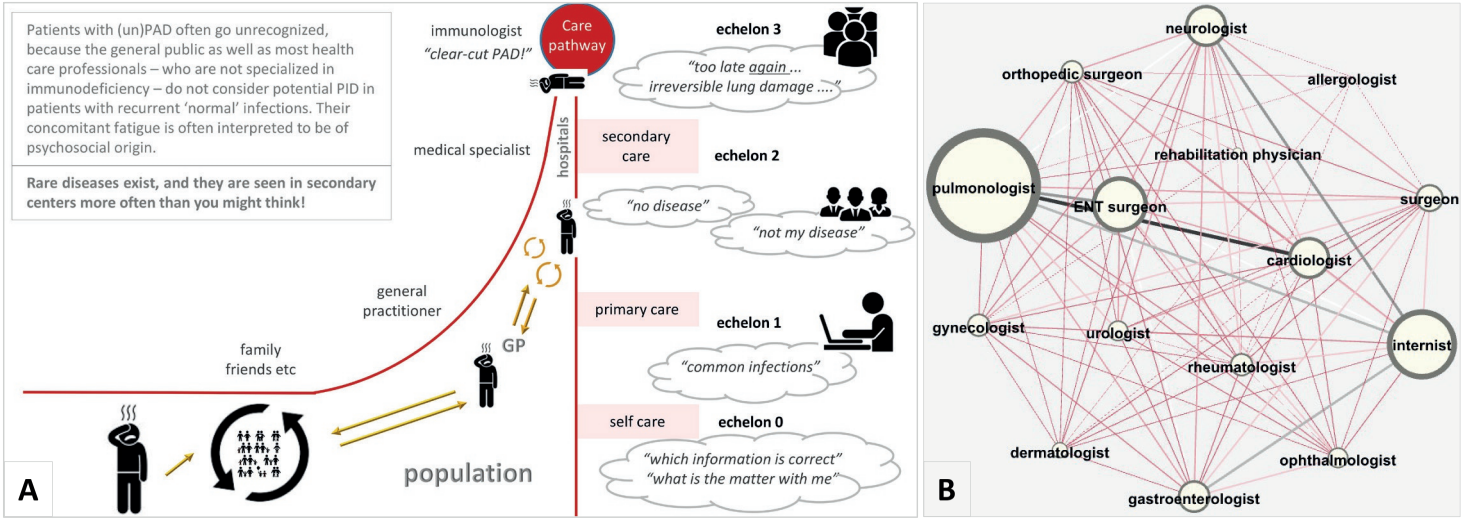


Figure 2.2. A. The long journey patients with (un)PAD often face toward diagnosis. B. The clinical specialists the 99 adult patients with PAD had visited prior to their referral to the Care Pathway.

Figure 2.2. The size of the nodes indicates the number of times the patients encountered that specialism and the size of the connecting lines between two nodes indicates the number of times that patients were known to both specialisms (created by Gephi software).

Abbreviations: ENT = ear-nose-throat specialist; GP = general practitioner; PAD = primary antibody deficiency.

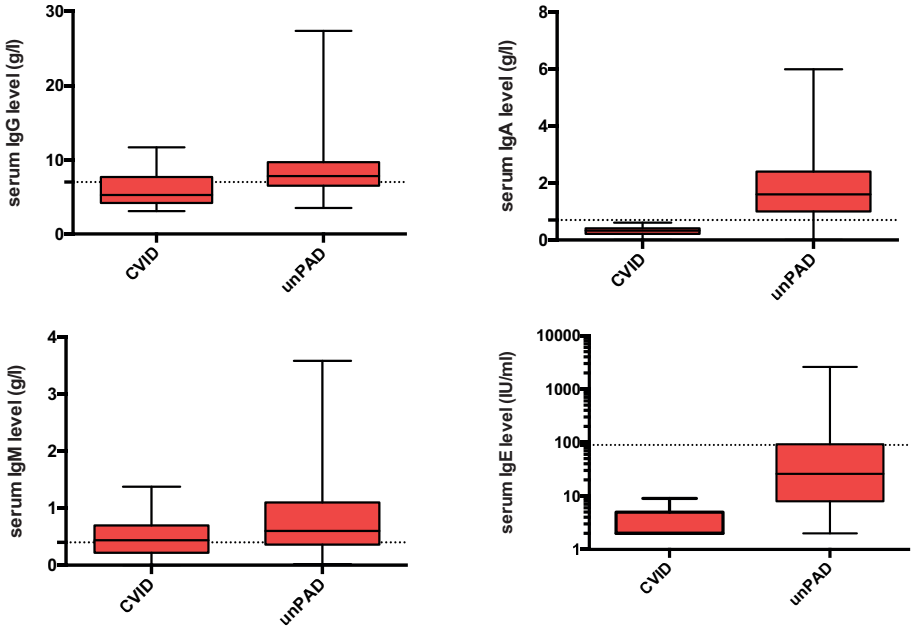


Figure 2.3. Mean serum immunoglobulins at diagnosis of the adults with CVID versus unPAD.

Figure 2.3. Abbreviations: CVID = common variable immunodeficiency disorder; unPAD = unclassified primary antibody deficiency [17].

The high prevalence of undetectable serum IgE in the CVID patients (57%, 4/7 patients) is in agreement with the recently published large CVID cohort by Lawrence et al [17], demonstrating that low/undetectable serum IgE is characteristic of CVID. Similar to the previously published European CVID cohort by Chapel et al. [18], the 89 unPAD patients were divided into categories based on presenting serum immunoglobulin levels (**Figure 2.4**). In most unPAD patients (62%) IgG and IgA levels were between 3.1 and 6.5 and > 0.8 g/l, respectively, while in the cohort of Chapel et al. the majority of patients (94.2%) had initial IgG levels <4.5 g/l at diagnosis [18].

Classification of the unPAD patients according to their immunoglobulin levels and pneumococcal vaccination response (PVR) is shown in **Supplementary Figures 2.1 and 2.2**. Median B and T lymphocyte counts were largely within the normal range (**Table 2.1**). A pulmonary HRCT scan was performed in 60 patients; of these, 53 had unPAD. Of the unPAD patients, 25 (47%) showed bronchial wall thickening, 24 (44%) bronchiectasis in one or more lobe(s), 11 (21%) central or peripheral mucus plugging, and 10 (19%) atelectasis. 20 patients were put on prophylactic co-trimoxazole (CVID, n=1; unPAD, n=19), 6 on subcutaneous and 23 on intravenous immunoglobulin substitution (CVID, n=9; unPAD, n=20).

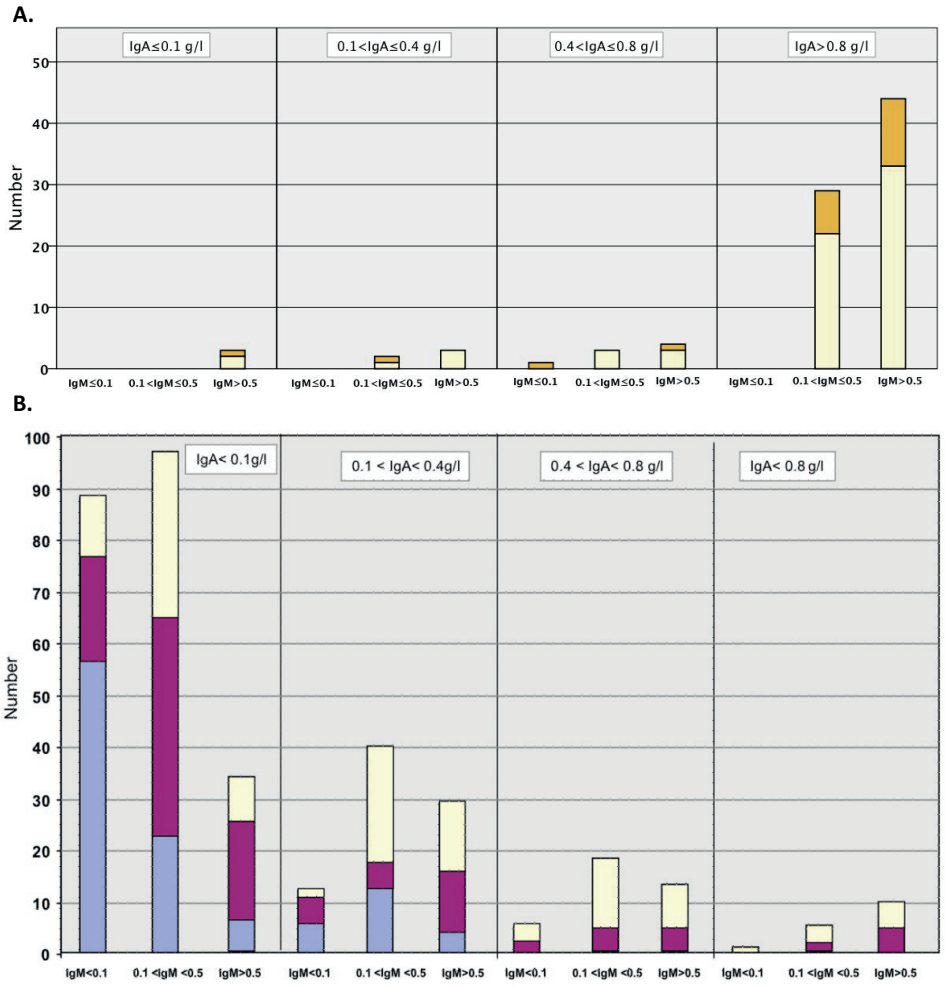


Figure 2.4. unPAD vs European CVID cohort.

Figure 2.4. A. unPAD cohort (89 patients): serum immunoglobulins (g/l) at diagnosis. Each column is divided into four parts, depending on the IgG levels; those in the light purple section with IgG ≤ 1.0 g/l; those in the dark purple section with IgG > 1.0 ≤ 3.0 g/l; those in the yellow section with IgG > 3.0 ≤ 6.5 g/l and those in the orange section with IgG > 6.5 g/l. B. For comparison: European CVID cohort (Chapel et al., 334 patients): serum immunoglobulins (g/l) at diagnosis. Unabridged from [18].

Table 2.1. Absolute number of lymphocyte subpopulations in peripheral blood in the unPAD patients.

Population	N=	Reference range x 10^e9/l[a]	Median (IQR) x 10^e9/l	Decreased (n)
Helper T-lymphocytes ($CD3^+CD4^+$)	75	0.5-2.0	0.9 (0.7-1.2)	4
T-lymphocytes ($CD3^+$)	75	0.78-3.0	1.4 (1.2-2.0)	4
Cytotoxic T-lymphocytes ($CD3^+CD8^+$)	75	0.2-1.2	0.5 (0.3-0.7)	3
NK-cells ($CD3^+CD16^+$and/or$CD56^+$)	74	0.10-1.2	0.2 (0.1-0.3)	4
B-lymphocytes ($CD19^+$)	75	0.064-0.82	0.20 (0.11-0.30)	3
IgM only memory B-lymphocytes ($CD19^+CD27^+IgM^+IgD^-$)	67	0.0011-0.015	0.0034 (0.0013-0.0066)	11
Switched memory B-lymphocytes ($CD19^+CD27^+IgM^-IgD^-$)	67	0.0045-0.13	0.021 (0.011-0.044)	6
CD21low B cells ($CD19^+CD21^{low}CD38^{low}$)	67	0.0017-0.049	0.0058 (0.003-0.010)	7[b]
Naïve B-lymphocytes ($CD19^+CD27^-IgM^+IgD^+$)	67	0.028-0.55	0.09 (0.046-0.16)	10
Transitional B cells ($CD19^+CD38^{++}IgM^{++}$)	67	0.0006-0.10	0.0082 (0.0031-0.016)	7

Table 2.1. [a]From Schatorjé et al., Age-matched Reference Values for B-lymphocyte Subpopulations and CVID Classifications in Children. Scand J Immunol 2011;74(5):502-10 [15]. *[b]Increased $CD21^{low}$ cell population in 2 patients.*

Statistical analyses in the adult patients

We assessed whether the concentrations of individual IgGsc correlated with the response to specific vaccine challenges (**Figure 2.5**). Spearman's correlations between IgGsc and PVR were moderate for IgG2 (r 0.52, 95% confidence interval (CI) 0.34-0.67, $p < 0.0001$), and weak for IgG4 (r 0.27, 95% CI 0.06-0.47, $p < 0.05$). There was a weak correlation between IgG1 and antigen-specific antibody response against tetanus toxoid (r 0.26, 95% CI 0.04-0.46, $p < 0.05$). Detailed results of all IgG subclasses plotted on a logarithmic scale with each PP serotype are shown in **Supplementary Figures 2.3 and 2.4**.

The combination of PVR, IgA and IgG at first presentation to the Pathway could not predict bronchiectasis in the adult PAD patients. Pulmonary HRCT scan findings were also not associated with referring doctor type, gender, the complaint 'being always ill', CVID vs. unPAD diagnosis, or type of prescribed therapy. Mucus plugging occurred significantly more often in patients with 'recurrent ENT and airway infections' and/or chronic cough (41%), compared to patients who were always tired or had other complaints (15%) (Fisher exact test [98], p=0.04).

9 CVID and 89 unPAD patients completed the online TAAQOL questionnaire. Patients with unPAD scored significantly worse (P< 0.01) on all domains compared to the subjects in the reference dataset (**Figure 2.6**). 3/9 CVID patients had already started immunoglobulin substitution when completing the TAAQOL questionnaire, therefore, it is impossible to draw conclusions from this group. In order to create separate subgroups of similar patients, associations between the key variables were examined. Cluster analysis showed that specifying two clusters resulted in the highest F-statistic (24.8). The two clusters varied significantly for all but fine motor functioning TAAQOL domain scores, but these clusters did not match the division between CVID and unPAD patients. The full results of the performed analyses can be found in **Supplementary Tables 2.3 and 2.4**.

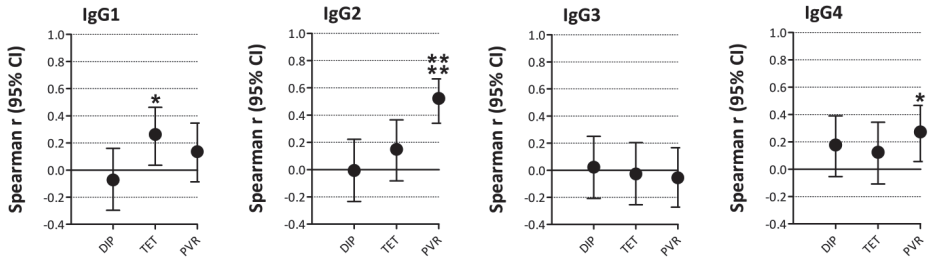


Figure 2.5. Correlations between vaccination responses and IgG-subclass levels.

*Figure 2.5. The graphs show the Spearman's correlation coefficients and 95% confidence interval (CI) for IgG1-4 vs. vaccination responses. *P< 0.05, **P< 0.01, ***P< 0.001, ****P< 0.0001.*

Abbreviations: DIP = diphtheria; TET = tetanus; PVR = pneumococcal vaccination response.

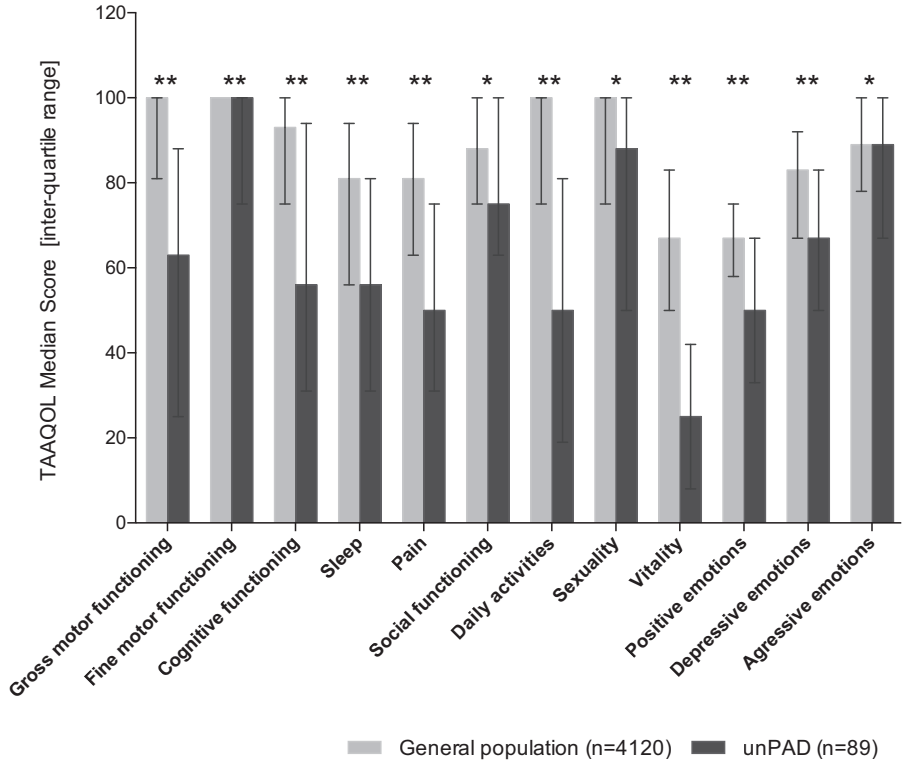


Figure 2.6. HR-QoL in 89 unPAD patients compared to the age-matched subjects from the reference dataset (n=4,120).

*Figure 2.6. Higher scores indicate better HR-QoL (TAAQOL); the maximum score is 100. Error bars indicate inter-quartile ratios. Statistically significant differences are indicated by an asterisk: *p < 0.01; **p < 0.001.*

Abbreviations: HR-QoL = health-related quality of life; TAAQOL = TNO-AZL questionnaire for adult's HRQoL; unPAD = unclassified primary antibody deficiency.

DISCUSSION

This description of patients with primary antibody deficiency (PAD) is unique in its secondary centre population and its focus on unPAD. It is also the first study to examine HRQoL in unPAD patients. Our results demonstrate that unPAD can result in severe patient burden. Besides the high proportion of patients suffering from bronchiectasis, their HRQoL was significantly impaired in all domains compared to the reference population. This study shows for the first time that many unPAD patients had to cope simultaneously with pain, negative feelings and impairments in cognition, home management tasks, sleep, social interaction, and work. A small number of previous studies have investigated HRQoL in PADs [7,9,19], some focusing solely on CVID [11,12,20,21], but patients with unPAD were not included in these studies. This unPAD group, generally considered to be a 'mild' form of hypogammaglobulinemia, has hardly received any attention in the literature [22]. Based on our results, we strongly recommend to change this.

The most severely affected HRQoL domain was vitality, indicating unPAD patients feel extremely tired and worn out. This parallels the above-average observed frequency of fatigue in PAD patients, not only compared to the general population, but also compared to the total PID population [23]. Fatigue is an important and debilitating problem, because it can lead to decreased daily activities, resulting in general deconditioning, which further affects fatigue and HRQoL in general. Clinicians should be aware of this.

While it was previously thought that bronchiectasis is the result of repeated infections due to deficient antibody production [24], there is increasing evidence that immune dysregulation plays an important role in the disease process [25]. Based on these new insights, the high proportion of unPAD patients suffering from bronchiectasis at presentation in our cohort (44%) – similar to the frequency reported in the literature for CVID patients [22,26–29] – is not that surprising. Clearly, despite the only moderately decreased immunoglobulin levels in unPAD compared to CVID patients, unPAD can result in comparable serious pulmonary complications.

Interestingly, there were nearly twice as many boys with unPAD in our paediatric cohort, but considerably more women than men in the adult group (74%). This may indicate that these diseases differ in different age groups. Perhaps unknown X-linked disease plays a role in some of these boys with antibody deficiency [30,31]. The female predominance in the adult unPAD patients suggests the pathogenesis of unPAD may differ between adult women and men. The tendency for immune dysregulation is widely acknowledged to be greater in women [32], but it is also possible that there are protective factors in men. It would be interesting to confirm this pattern in a much larger cohort, and to further explore potential gender-specific mechanisms.

IgG-subclass measurements could not predict pneumococcal vaccination responses (PVRs), or vice versa. Only a moderate correlation between IgG2-subclasses and PVRs was found. These results are in agreement with previous studies in patients with Hodgkin's lymphoma [33], in children with chronic chest infections [34], and in patients with IgA deficiency [35]. Thus, both are needed to fully explore the immune status of an individual patient.

Our study has several limitations. First, the sample size was limited. Therefore, the insignificant results of the exploration of distinct subgroups in the unPAD cohort or differences between the unPAD and CVID patients, might be caused by the limited detection power. A future study in a much larger cohort may well be able to reveal separate clinical entities; the ESID online Registry would be a good tool for this. Second, the few CVID patients had partly already started their immunoglobulin substitution therapy. This means our study should be mainly used as a thorough description of the – to date – largest cohort of unPAD patients, not as an important source for comparison of unPAD with CVID. Third, genetic testing was not performed in most patients; it would be interesting to investigate this. It is possible that mildly affected patients with a known genetic defect are 'hidden' in this cohort. Despite these limitations, our data show, contrary to what is currently assumed by most immunologists in specialized/tertiary centres, that 'mild' hypogammaglobulinemia can be a serious condition. Also for those patients, early detection and adequate treatment is important.

REFERENCES

1. Bousfiha AA, Jeddane L, Ailal F, Al Herz W, Conley ME, Cunningham-Rundles C, et al. A phenotypic approach for IUIS PID classification and diagnosis: guidelines for clinicians at the bedside. J Clin Immunol. 2013 Aug;33(6):1078–87.
2. Picard C, Al-Herz W, Bousfiha A, Casanova J-L, Chatila T, Conley ME, et al. Primary Immunodeficiency Diseases: an Update on the Classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015. J Clin Immunol. 2015 Nov;35(8):696–726.
3. De Vries E. Patient-centred screening for primary immunodeficiency: A multi-stage diagnostic protocol designed for non-immunologists. Clin Exp Immunol. 2006;
4. Gathmann B, Grimbacher B, Beaute J, Dudoit Y, Mahlaoui N, Fischer A, et al. The European internet-based patient and research database for primary immunodeficiencies: results 2006-2008. Clin Exp Immunol. 2009 Sep;157 Suppl:3–11.
5. Chapel H, Cunningham-Rundles C. Update in understanding common variable immunodeficiency disorders (CVIDs) and the management of patients with these conditions. Br J Haematol. 2009 Jun;145(6):709–27.
6. Jonkman-Berk BM, van den Berg JM, Ten Berge IJM, Bredius RGM, Driessen GJ, Dalm VASH, et al. Primary immunodeficiencies in the Netherlands: national patient data demonstrate the increased risk of malignancy. Clin Immunol. 2015 Feb;156(2):154–62.
7. Aghamohammadi A, Montazeri A, Abolhassani H, Saroukhani S, Pourjabbar S, Tavassoli M, et al. Health-related quality of life in primary antibody deficiency. Iran J Allergy Asthma Immunol. 2011 Mar;10(1):47–51.
8. Sigstad HMH, Stray-Pedersen A, Froland SS. Coping, quality of life, and hope in adults with primary antibody deficiencies. Health Qual Life Outcomes. 2005 May;3:31.
9. Titman P, Allwood Z, Gilmour C, Malcolmson C, Duran-Persson C, Cale C, et al. Quality of life in children with primary antibody deficiency. J Clin Immunol. 2014 Oct;34(7):844–52.
10. Jorgensen GH, Gardulf A, Sigurdsson MI, Arnlaugsson S, Hammarstrom L, Ludviksson BR. Health-related quality of life (HRQL) in immunodeficient adults with selective IgA deficiency compared with age- and gender-matched controls and identification of risk factors for poor HRQL. Qual Life Res. 2014 Mar;23(2):645–58.
11. Rider NL, Kutac C, Hajjar J, Scalchunes C, Seeborg FO, Boyle M, et al. Health-Related Quality of Life in Adult Patients with Common Variable Immunodeficiency Disorders and Impact of Treatment. J Clin Immunol. 2017 Jul;37(5):461–75.
12. Quinti I, Di Pietro C, Martini H, Pesce AM, Lombardi F, Baumghartner M, et al. Health related quality of life in common variable immunodeficiency. Yonsei Med J. 2012 May;53(3):603–10.
13. de Vries E. Patient-centred screening for primary immunodeficiency, a multi-stage diagnostic protocol designed for non-immunologists: 2011 update. Clin Exp Immunol. 2012 Jan;167(1):108–19.
14. Bruil, J, Fekkes, M, Vogels, T, Verrips G. No Title. TAAQOL Manual. Leiden Center for Child and Pediatrics LUMC-TNO. 2004.
15. Schatorje EJH, Gemen EFA, Driessen GJA, Leuvenink J, van Hout RWNM, van der Burg M, et al. Age-matched reference values for B-lymphocyte subpopulations and CVID classifications in children. Scand J Immunol. 2011 Nov;74(5):502–10.
16. Edgar D, Ehl S. ESID Registry - Working definitions for clinical diagnosis of PID. 2016;1–9.

17. Lawrence MG, Palacios-Kibler T V, Workman LJ, Schuyler AJ, Steinke JW, Payne SC, et al. Low Serum IgE Is a Sensitive and Specific Marker for Common Variable Immunodeficiency (CVID). J Clin Immunol. 2018 Apr;38(3):225–33.
18. Chapel H, Lucas M, Lee M, Bjorkander J, Webster D, Grimbacher B, et al. Common variable immunodeficiency disorders: division into distinct clinical phenotypes. Blood. 2008 Jul;112(2):277–86.
19. Ataeinia B, Montazeri A, Tavakol M, Azizi G, Kiaee F, Tavakolinia N, et al. Measurement of Health-Related Quality of Life in Primary Antibody-Deficient Patients. Immunol Invest. 2017 May;46(4):329–40.
20. Tcheurekdjian H, Palermo T, Hostoffer R. Quality of life in common variable immunodeficiency requiring intravenous immunoglobulin therapy. Ann Allergy Asthma Immunol. 2004 Aug;93(2):160–5.
21. Tabolli S, Giannantoni P, Pulvirenti F, La Marra F, Granata G, Milito C, et al. Longitudinal study on health-related quality of life in a cohort of 96 patients with common variable immune deficiencies. Front Immunol. 2014;5:605.
22. Driessen GJ, Dalm VASH, van Hagen PM, Grashoff HA, Hartwig NG, van Rossum AMC, et al. Common variable immunodeficiency and idiopathic primary hypogammaglobulinemia: two different conditions within the same disease spectrum. Haematologica. 2013 Oct;98(10):1617–23.
23. Hajjar J, Guffey D, Minard CG, Orange JS. Increased Incidence of Fatigue in Patients with Primary Immunodeficiency Disorders: Prevalence and Associations Within the US Immunodeficiency Network Registry. J Clin Immunol. 2017 Feb;37(2):153–65.
24. Maglione PJ. Autoimmune and Lymphoproliferative Complications of Common Variable Immunodeficiency. Curr Allergy Asthma Rep. 2016 Mar;16(3):19.
25. Boyton RJ, Altmann DM. Bronchiectasis: Current Concepts in Pathogenesis, Immunology, and Microbiology. Annu Rev Pathol. 2016 May;11:523–54.
26. Bazregari S, Azizi G, Tavakol M, Asgardoon MH, Kiaee F, Tavakolinia N, et al. Evaluation of infectious and non-infectious complications in patients with primary immunodeficiency. Cent J Immunol. 2017;42(4):336–41.
27. Westh L, Mogensen TH, Dalgaard LS, Bernth Jensen JM, Katzenstein T, Hansen A-BE, et al. Identification and Characterization of a Nationwide Danish Adult Common Variable Immunodeficiency Cohort. Scand J Immunol. 2017 Jun;85(6):450–61.
28. Yong PFK, Thaventhiran JED, Grimbacher B. "A rose is a rose is a rose," but CVID is Not CVID common variable immune deficiency (CVID), what do we know in 2011? Adv Immunol. 2011;111:47–107.
29. Verma N, Grimbacher B, Hurst JR. Lung disease in primary antibody deficiency. Lancet Respir Med. 2015 Aug;3(8):651–60.
30. Schatorje EJH, de Jong E, van Hout RWNM, Garcia Vivas Y, de Vries E. The Challenge of Immunoglobulin-G Subclass Deficiency and Specific Polysaccharide Antibody Deficiency--a Dutch Pediatric Cohort Study. J Clin Immunol. 2016 Feb;36(2):141–8.
31. Schatorje EJH, Gathmann B, van Hout RWNM, de Vries E. The PedPAD study: boys predominate in the hypogammaglobulinaemia registry of the ESID online database. Clin Exp Immunol. 2014 Jun;176(3):387–93.
32. Quintero OL, Amador-Patarroyo MJ, Montoya-Ortiz G, Rojas-Villarraga A, Anaya J-M. Autoimmune disease and gender: plausible mechanisms for the female predominance of autoimmunity. J Autoimmun. 2012 May;38(2–3):J109-19.

33. Siber GR, Schur PH, Aisenberg AC, Weitzman SA, Schiffman G. Correlation between serum IgG-2 concentrations and the antibody response to bacterial polysaccharide antigens. N Engl J Med. 1980 Jul;303(4):178–82.
34. Smith TF, Bain RP, Schiffman G. Relationship between serum IgG2 concentrations and antibody responses to pneumococcal polysaccharides in children with chronic chest symptoms. Clin Exp Immunol. 1990 Jun;80(3):339–43.
35. Edwards E, Razvi S, Cunningham-Rundles C. IgA deficiency: clinical correlates and responses to pneumococcal vaccine. Clin Immunol. 2004 Apr;111(1):93–7.

Supplementary Table 2.1. The children with primary antibody deficiency. Comprehensive overview of clinical and laboratory data of the 23 children (<18yrs at referral) with PAD who visited the Care Path February 2012 - June 2016 (inclusive) and for whom informed consent for inclusion in this study was obtained.

= *THE CHILDREN*	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
gender	M	M	M	M	F	F	M	M	M	M
age at referral (yrs)	13	13	13	13	10	9	8	9	7	7
first clinical presentation[1]	res	res	res	res	res	res	res	ftt	pyo	res
ESID Registry working diagnosis[2]	cvid	uAD	uAD	uAD	cvid	cvid	uAD	sIgM	uAD	uAD
additional diagnostic information	iron	-	-	-	-	-	iron	eci	eci	iron
familial case	N	Y	N	N	Y	Y	Y	N	N	N
HRCT scan lungs[3]	nml	brp	-	-	brp	nml	-	-	-	nml
therapy (related to ID)	sc	-	-	ab	sc	iv	-	-	-	iv
IgG (g/l)[4]	5.8 (5,2-15,6)	4.9 (5,2-15,6)	10.9 (5,2-15,6)	8.1 (5,2-15,6)	5.1 (5,2-15,6)	4.4 (5,2-15,6)	7.2 (5,2-15,6)	9.9 (5,2-15,6)	9.1 (5,2-15,6)	3.6 (5,2-15,6)
IgG1 (g/l)[4]	5.3 (3,7-12,8)	2.8 (3,7-12,8)	8.1 (3,7-12,8)	6.1 (3,7-12,8)	3.8 (3,7-12,8)	2.8 (3,7-12,8)	5.1 (3,7-12,8)	-	6.8 (3,7-12,8)	2,8 (3,7-12,8)
IgG2 (g/l)[4]	0.40 (0,85-6,1)	1.70 (0,85-6,1)	1.07 (0,85-6,1)	0.84 (0,85-6,1)	0.96 (0,85-6,1)	0.98 (0,85-6,1)	1.25 (0,85-6,1)	-	1.28 (0,85-6,1)	0.76 (0,85-6,1)
IgG3 (g/l)[4]	0.16 (0,13-1,63)	0.74 (0,13-1,63)	0.16 (0,13-1,63)	0.61 (0,13-1,63)	0.68 (0,13-1,63)	0.21 (0,13-1,63)	0.22 (0,13-1,63)	-	0.12 (0,13-1,63)	0.19 (0,13-1,63)
IgG4 (g/l)[4]	0.029 (0,023-2,3)	0.235 (0,023-2,3)	0.146 (0,023-2,3)	0.012 (0,023-2,3)	0.221 (0,023-2,3)	0.019 (0,023-2,3)	0.419 (0,023-2,3)	-	0.062 (0,023-2,3)	0.065 (0,023-2,3)
IgA (g/l)[4]	0.50 (0,54-3,6)	0.83 (0,54-3,6)	1.07 (0,54-3,6)	2.35 (0,54-3,6)	0.28 (0,54-3,6)	0.28 (0,54-3,6)	0.08 (0,54-3,6)	1.28 (0,54-3,6)	0.92 (0,54-3,6)	0.61 (0,54-3,6)
IgM (g/l)[4]	0.21 (0,31-2,4)	0.39 (0,31-2,4)	0.79 (0,31-2,4)	0.83 (0,31-2,4)	0.57 (0,31-2,4)	0.47 (0,31-2,4)	0.73 (0,31-2,4)	0.22 (0,31-2,4)	0.87 (0,31-2,4)	0.99 (0,31-2,4)
$CD3^+CD4^+$ Th ($x10^9$/l)[4]	1.00 (0,5-1,3)	0.89 (0,5-1,3)	-	0.85 (0,5-1,3)	1.20 (0,5-1,8)	0.70 (0,5-1,8)	0.70 (0,5-1,8)	0.66 (0,5-1,8)	-	1.32 (0,5-1,8)
$CD19^+$ B ($x10^9$/l)[4]	0.20 (0,2-0,5)	0.56 (0,2-0,5)	-	0.63 (0,2-0,5)	0.60 (0,3-0,7)	0.30 (0,3-0,7)	0.20 (0,3-0,7)	0.23 (0,3-0,7)	-	0.92 (0,3-0,7)
B21lo ($x10^9$/l)[4]	0.0019 (0,0039-0,037)	0.0008 (0,0039-0,037)	-	0.0038 (0,0039-0,037)	0.0073 (0,0059-0,036)	0.0176 (0,0059-0,036)	0.0200 (0,0059-0,036)	0.0033 (0,0059-0,036)	-	0.0026 (0,0059-0,036)
smB ($x10^9$/l)[4]	0.0014 (0,0065-0,073)	0.0225 (0,0065-0,073)	-	0.0152 (0,0065-0,073)	0.0569 (0,0070-0,051)	0.0149 (0,0070-0,051)	0.0100 (0,0070-0,051)	0.0226 (0,0070-0,051)	-	0.0336 (0,0070-0,051)

	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23
	M	M	M	F	M	M	M	M	F	F	M	F	M
	6	6	6	4	5	6	4	16	4	3	2	5	3
	res	res	res	un	res	ftt	ail	res	res	res	res	res	res
	uAD	uAD	uAD	uAD	THI	uAD	uAD	sIgA	uAD	THI	uAD	sIgA	uAD
	-	iron	iron	shingles	iron	iron	cITP MBL	-	iron	BHR	-	-	iron
	N	N	N	N	N	N	N	N	N	N	N	Y	N
	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	fe	ab	-	fe	fe	-	-	fe	ab	-	ab	ab
	11.4 (4,3-13,4)	5.0 (4,3-13,4)	9.0 (4,3-13,4)	6.8 (4,3-13,4)	5.2 (4,3-13,4)	8.3 (4,3-13,4)	5.6 (4,3-13,4)	12.8 (7,0-16,0)	4.5 (4,3-13,4)	5.2 (4,3-13,4)	6.4 (4,3-13,4)	16.0 (4,3-13,4)	8.2 (4,3-13,4)
	9.3 (3,2-10,0)	4.3 (3,2-10,0)	7.0 (3,2-10,0)	5.2 (3,2-10,0)	3.7 (3,2-10,0)	7.4 (3,2-10,0)	4.5 (3,2-10,0)	8.5 (3,7-12,8)	3.9 (3,2-10,0)	3.4 (3,2-10,0)	5.1 (3,2-10,0)	11.4 (3,2-10,0)	6.5 (3,2-10,0)
	0.57 (0,52-3,4)	0.79 (0,52-3,4)	0.57 (0,52-3,4)	0.62 (0,52-3,4)	0.68 (0,52-3,4)	0.59 (0,52-3,4)	0.65 (0,52-3,4)	2.12 (0,85-6,1)	0.14 (0,52-3,4)	1.44 (0,52-3,4)	0.77 (0,52-3,4)	3.57 (0,52-3,4)	0.93 (0,52-3,4)
	0.81 (0,13-1,33)	0.23 (0,13-1,33)	0.22 (0,13-1,33)	0.15 (0,13-1,33)	0.17 (0,13-1,33)	0.20 (0,13-1,33)	0.25 (0,13-1,33)	0.50 (0,13-1,63)	0.04 (0,13-1,33)	0.22 (0,13-1,33)	0.14 (0,13-1,33)	0.80 (0,13-1,33)	0.49 (0,13-1,33)
	0.367 (0,012-1,58)	0.039 (0,012-1,58)	0.065 (0,012-1,58)	0.160 (0,012-1,58)	0.526 (0,012-1,58)	0.028 (0,012-1,58)	0.014 (0,012-1,58)	0.228 (0,023-2,3)	0.044 (0,012-1,58)	0.171 (0,012-1,58)	0.105 (0,012-1,58)	0.668 (0,012-1,58)	0.073 (0,012-1,58)
	1.00 (0,19-2,2)	0.31 (0,19-2,2)	0.89 (0,19-2,2)	0.52 (0,19-2,2)	0.86 (0,19-2,2)	1.29 (0,19-2,2)	0.67 (0,19-2,2)	0.25 (0,70-4,0)	0.40 (0,19-2,2)	0.25 (0,19-2,2)	0.45 (0,19-2,2)	0.00 (0,19-2,2)	0.44 (0,19-2,2)
	0.95 (0,21-1,8)	0.61 (0,21-1,8)	1.15 (0,21-1,8)	0.52 (0,21-1,8)	0.77 (0,21-1,8)	1.63 (0,21-1,8)	0.63 (0,21-1,8)	0.44 (0,40-2,3)	0.27 (0,21-1,8)	0.20 (0,21-1,8)	0.42 (0,21-1,8)	0.83 (0,21-1,8)	1.20 (0,21-1,8)
	0.70 (0,5-1,8)	-	-	-	-	-	0.87 (0,7-2,0)	-	1.26 (0,7-2,0)	-	1.5 (0,7-2,0)	1.57 (0,7-2,0)	-
	0.70 (0,3-0,7)	-	-	-	-	-	0.60 (0,4-1,5)	-	0.44 (0,4-1,5)	-	0.60 (0,4-1,5)	0.51 (0,4-1,5)	-
	0.0106 (0,0059-0,036)	-	-	-	-	-	0.0029 (0,0069-0,099)	-	0.0037 (0,0069-0,099)	-	-	0.0126 (0,0069-0,099)	-
	0.0365 (0,0070-0,051)	-	-	-	-	-	0.0167 (0,0022-0,25)	-	0.0134 (0,0022-0,25)	-	-	0.0262 (0,0022-0,25)	-

Supplementary Table 2.1. Continued.

= *THE CHILDREN*	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
granulocytes	nml nr	nml nr	nml nr	nml nr	nml nr	nml nr	nml nF	nml nr	nml nF	nml nF
classical C pathway	nml	nml	nml	-	nml	nml	nml	-	nml	Nml
alternative C pathway	nml	nml	nml	-	nml	nml	nml	-	nml	Nml
MBL C pathway	-	nml	-	-	-	-	-	-	-	-
ANA (titer)	>1:640	neg	-	-	neg	1:160	neg	-	-	neg
Ferritin (nml: 25-250mg/l)	15	35	-	-	nml	nml	18	-	48	13
IgE (IU/ml) (nml: <50 ≤10yrs; <100 >10yrs)	48	16	350	-	6	22	33	130	110	53
sIgE(s)	neg	neg	neg	neg	neg	neg	neg	neg	neg	pos
response diphtheria[5]	0.53	1.17	-	0.04	1.29	0.22	0.08	-	4.41	1.72
response tetanus[5]	>16	>16	-	0.82	4.03	0.55	2.15	-	12.38	7.17
response Pneumo23[6]	abs	nml	-	snml	snml	snml	snml	-	nml	snml
QoL-parents body[7]	28	-	26	26	26	27	28	28	28	27
QoL-child body[7]	26	-	18	17	12	28	23	25	-	-
reference p10-p90[7]	16-30	-	16-30	16-30	18-31	18-31	18-31	18-31	-	-
QoL-parents motor[7]	28	-	31	32	30	30	32	27	30	29
QoL-child motor[7]	24	-	32	30	15	27	30	25	-	-
reference p10-p90[7]	26-32	-	26-32	26-32	26-32	26-32	26-32	26-32	-	-
QoL-parents cogn[7]	25	-	28	27	30	28	32	25	28	31
QoL-child cogn[7]	26	-	23	30	25	27	32	31	-	-
reference p10-p90[7]	22-32	-	22-32	22-32	23-32	23-32	23-32	23-32	-	-
QoL-parents social[7]	32	-	32	32	32	32	30	32	32	30
QoL-child social[7]	32	-	32	32	32	32	30	32	-	-
reference p10-p90[7]	29-32	-	29-32	29-32	26-32	26-32	26-32	26-32	-	-
QoL-parents pos[7]	12	-	16	16	9	15	15	14	16	14
QoL-child pos[7]	13	-	16	16	9	16	15	15	-	-
reference p10-p90[7]	9-16	-	9-16	9-16	10-16	10-16	10-16	10-16	-	-
QoL-parents neg[7]	12	-	14	15	4	12	11	16	14	9
QoL-child neg[7]	13	-	14	16	4	13	11	13	-	-
reference p10-p90[7]	8-15	-	8-15	8-15	8-15	8-15	8-15	8-15	-	-
QoL stomach[8]	-	-	-	-	-	-	-	-	-	-
reference p10-p90[8]	-	-	-	-	-	-	-	-	-	-
QoL skin[8]	-	-	-	-	-	-	-	-	-	-
reference p10-p90[8]	-	-	-	-	-	-	-	-	-	-
QoL lung[8]	-	-	-	-	-	-	-	-	-	-
reference p10-p90[8]	-	-	-	-	-	-	-	-	-	-
QoL sleeping[8]	-	-	-	-	-	-	-	-	-	-
reference p10-p90[8]	-	-	-	-	-	-	-	-	-	-
QoL appetite[8]	-	-	-	-	-	-	-	-	-	-
reference p10-p90[8]	-	-	-	-	-	-	-	-	-	-
QoL lively[8]	-	-	-	-	-	-	-	-	-	-

C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23
nml nr	nml nr	nml nr	nml nr	nml nr	nml nr	nml nr	nml nr	nml nr	nml nr	nml nF	nml nr	nml nr
nml	nml	nml	-	nml	nml	nml	nml	nml	nml	-	nml	nml
nml	nml	nml	-	nml	nml	nml	nml	nml	nml	-	nml	nml
-	-	nml	-	nml	nml	snml	nml	-	-	-	nml	nml
-	-	neg	neg	-	neg	-	-	-	-	-	neg	-
48	9.4	9	50	16	11	-	78	14	-	-	44	21
23	92	5	61	6	4	1100	24	24	40	410	460	19
neg	neg	neg	neg	neg	-	pos	neg	neg	neg	neg	pos	neg
1.45	5.24	-	-	1.89	0.18	0.89	-	0.16	1.65	-	0.71	0.81
14.46	12.84	-	-	2.08	1.01	13.19	-	6.97	2.04	-	1.56	1.17
nml	nml	nml	-	nml	nml	nml	-	nml	nml	-	nml	snml
26	30	28	-	-	27	-	29	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	16-30	-	-	-	-	-
31	32	32	-	-	24	-	32	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	26-32	-	-	-	-	-
32	30	31	-	-	32	-	30	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	22-32	-	-	-	-	-
30	32	32	-	-	28	-	32	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	29-32	-	-	-	-	-
14	16	15	-	-	15	-	16	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	9-16	-	-	-	-	-
9	14	11	-	-	10	-	10	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	8-15	-	-	-	-	-
-	-	-	50	100	-	92	-	67	100	33	50	67
-	-	-	73-100	73-100	-	73-100	-	73-100	73-100	73-100	73-100	73-100
-	-	-	75	100	-	75	-	92	100	100	100	33
-	-	-	75-100	75-100	-	75-100	-	75-100	75-100	75-100	75-100	75-100
-	-	-	100	67	-	50	-	100	100	50	67	67
-	-	-	75-100	75-100	-	75-100	-	75-100	75-100	75-100	75-100	75-100
-	-	-	100	100	-	100	-	94	88	38	88	81
-	-	-	56-100	56-100	-	56-100	-	56-100	56-100	56-100	56-100	56-100
-	-	-	92	67	-	100	-	67	75	33	92	58
-	-	-	75-100	75-100	-	75-100	-	75-100	75-100	75-100	75-100	75-100
-	-	-	50	67	-	100	-	100	100	67	100	67

Supplementary Table 2.1. Continued.

= *THE CHILDREN*	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
reference p10-p90[8]	-	-	-	-	-	-	-	-	-	-
QoL pos[8]	-	-	-	-	-	-	-	-	-	-
reference p10-p90[8]	-	-	-	-	-	-	-	-	-	-
QoL probl behav[8]	-	-	-	-	-	-	-	-	-	-
reference p10-p90[8]	-	-	-	-	-	-	-	-	-	-
QoL anxiety[8]	-	-	-	-	-	-	-	-	-	-
reference p10-p90[8]	-	-	-	-	-	-	-	-	-	-
QoL social[8]	-	-	-	-	-	-	-	-	-	-
reference p10-p90[8]	-	-	-	-	-	-	-	-	-	-
QoL motor[8]	-	-	-	-	-	-	-	-	-	-
reference p10-p90[8]	-	-	-	-	-	-	-	-	-	-
QoL communication[8]	-	-	-	-	-	-	-	-	-	-
reference p10-p90[8]	-	-	-	-	-	-	-	-	-	-

Supplementary Table 2.1. [1] *According to the eight clinical presentations of primary immunodeficiency described in reference (1).* [2] *According to the working diagnoses used in the ESID online Registry described in reference (2). For sIgA and sIgM, the absence of clinical signs of T-cell deficiency was considered sufficient. When taking all details of the ESID Registry Working Diagnoses into account, sIgA, sIgM and uAD in these patients all fall under the Working Diagnosis 'unclassified antibody deficiency' (unPAD).* [3] *Prior to or no immunoglobulin substitution.* [4] *Age-related reference values in brackets, according to reference (1).* [5] *Response to vaccination with diphtheria-tetanus-poliomyelitis booster vaccination was determined (pre-post vaccination titres in IU/ml).* [6] *Response to vaccination with Pneumo23 polysaccharide 23-valent pneumococcal vaccination (normal[nml], subnormal[snml], absent[abs]; according to the reference values of the laboratory doing the tests, only serotypes not influenced by protein-conjugated vaccination received previously are used for the evaluation of the results).* [7] *Determined using the TNO Quality of Life Questionnaires for children TAC-QOL parent resp. child questionnaire, 10th-90th centile values of the Dutch age-related reference population (8-11 and 12-15yrs resp.)(3); body = problems /limitations concerning general physical functioning/complaints; motor = problems / limitations concerning motor functioning; auto = problems / limitations concerning independent daily functioning; cogn = problems / limitations concerning cognitive functioning and school performances; social = problems / limitations in social contacts, with parents and peers; pos = the occurrence of positive moods; neg = the occurrence of negative moods.* [8] *Determined using the TNO Quality of Life Questionnaires for children TAP-QOL questionnaire, 10th-90th centile values of the Dutch age-related reference population(4); stomach = measures stomach and intestinal problems; skin = measures skin problems like eczema, itchiness, and dry skin; lung = measures difficulties with breathing, lung problems, bronchitis or shortness of breath; sleeping = measures sleeping problems like being awake or crying or difficulty sleeping during the night; appetite = measures if the child had a bad appetite, difficulty to eat enough of refused to eat; probl behav = measures difficult and aggressive behaviour of the child; pos = measures positive emotions; anxiety = measures if the child was anxious, tense or frightened; lively = measures if the child was active, lively and*

C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23
-	-	-	100-100	100-100	-	100-100	-	100-100	100-100	100-100	100-100	100-100
-	-	-	100	100	-	100	-	100	100	83	100	100
-	-	-	100-100	100-100	-	100-100	-	100-100	100-100	100-100	100-100	100-100
-	-	-	57	79	-	93	-	71	71	50	50	57
-	-	-	50-86	50-86	-	50-86	-	50-86	50-86	50-86	50-86	50-86
-	-	-	50	50	-	67	-	67	83	100	67	67
-	-	-	50-100	50-100	-	50-100	-	50-100	50-100	50-100	50-100	50-100
-	-	-	100	100	-	100	-	100	100	100	100	100
-	-	-	67-100	67-100	-	67-100	-	67-100	67-100	67-100	67-100	67-100
-	-	-	100	88	-	100	-	100	100	100	100	100
-	-	-	94-100	94-100	-	94-100	-	94-100	94-100	94-100	94-100	94-100
-	-	-	81	88	-	100	-	81	100	81	100	88
-	-	-	81-100	81-100	-	81-100	-	81-100	81-100	81-100	81-100	81-100

energetic; social = measures social contacts with other children, like if the child was at ease with other children; motor = measures gross motor problems like difficulties with walking, running, climbing stairs and balance; communication = measures communicative skills of the child when compared to children of the same age. Abbreviations: red = value outside age-related reference; - = not available, not performed; ab = daily antibiotic prophylaxis; ail = the clinical presentation 'autoimmune or chronic inflammatory disease; lymphoproliferation'; ANA = antinuclear antibody; B = B-lymphocytes; B21lo = $CD19^{+}CD38^{low}CD21^{low}$ B-lymphocytes; BHR = bronchial hyperreactivity; brp = bronchopathy (according to the attending radiologist); C = complement; C1 = child with study number 1; CD = cluster of differentiation; cITP = chronic immune thrombocytopenic purpura; eci = 'e causa ignota' (cause not found); ENT = ear-nose-throat; F = female; fe = 3 months of oral iron supplementation; ftt = the clinical presentation 'failure to thrive from early infancy'; g/l = gram per liter; HRCT = high resolution computerized tomography; Ig = immunoglobulin; iron = deficient stores (low ferritin); IU/ml = international units per millilitre; iv = intravenous immunoglobulin substitution; lo = low (surface expression); lym = lymphocytes; M = male; MBL = mannose binding lectin deficiency; ml = milliliter; N = no; neg = negative; nf = number + function; nml = normal; nr = number; PAD = primary antibody deficiency; pos = positive; pyo = the clinical presentation 'recurrent pyogenic infections'; res = the clinical presentation 'recurrent ENT and airway infections'; sc = subcutaneous immunoglobulin substitution; sIgA = selective IgA deficiency (absence of clinical signs of T-cell deficiency was considered sufficient); sIgE(s) = positive specific IgE antibody/antibodies in serum; sIgM = selective IgM deficiency (absence of clinical signs of T-cell deficiency was considered sufficient); smB = switched memory B-lymphocytes ($CD19^{+}CD27^{+}IgD^{-}IgM^{-}$ lymphocytes); snml = subnormal (but not absent); Th = T-helper lymphocytes; THI = the clinical presentation 'transient hypogammaglobulinemia of infancy' (the immunological abnormalities resolved during the study); uAD = unclassified antibody deficiency; un = the clinical presentation 'unusual infections or unusually severe course of infections'; Y = yes; yrs = years.

REFERENCES

1. de Vries E. Patient-centred screening for primary immunodeficiency, a multi-stage diagnostic protocol designed for non-immunologists: 2011 update. Clin Exp Immunol. 2012 Jan;167(1):108–19.
2. Edgar D, Ehl S. ESID Registry - Working definitions for clinical diagnosis of PID. 2016;1–9.
3. Nederlandse D. Vragenlijsten kwaliteit van leven.
4. TACQOL CF 8-11 Reference data. https://www.tno.nl/nl/aandachtsgebieden/gezond-leven/prevention-work-health/gezond-en-veilig-opgroeien/vragenlijsten-gezondheidsrelateerde-kwaliteit-van-leven/

Supplementary Table 2.2. Descriptive statistics in 99 adults with primary antibody deficiency.

Continuous variables			
Variable	*Number of responses*	*Mean*	*Standard deviation*
BMI	95	26.4	5.1
Smoking pack years	97	7	0.20

Categorical variables			
Variable	*Category*	*Number*	*Percentage*
Referred by	Allergologist	1	1%
	ENT	2	2%
	Gastroenterologist	1	1%
	General Practitioner	35	35%
	Internist	15	15%
	Paediatrician	2	2%
	Pulmonologist	43	43%
Diagnosis	CVID	10	10%
	Selective IgA deficiency	4	4%
	Selective IgM deficiency	4	4%
	Unclassified antibody deficiency	81	82%
HRCT bronchial wall thickening	No	31	52%
	Yes	29	48%
HRCT bronchiectasis	None	31	52%
	1 lobe	9	15%
	2 / 3 lobes	10	17%
	4+ lobes	10	17%
HRCT mucus plugging	None	49	82%
	Central	3	5%
	Peripheral	8	13%
Atelectasis	No	50	83%
	Yes	10	17%
First clinical presentation	Autoimmunity, chronic inflammation	10	10%
	ENT-airway infections	88	89%
	Unusual infections	1	1%
Prescribed therapy (related to antibody deficiency diagnosis)	None	50	51%
	Prophylactic antibiotics	20	20%
	IVIG	23	23%
	SCIG	6	6%
Familial case	No	79	80%
	Yes	20	20%
Gender	Male	28	28%
	Female	71	72%

Supplementary Table 2.2. Continued.

Highest education level	None	21	22%
	VMBO (pre-MBO)	17	18%
	HAVO (pre-HBO)	5	5%
	MBO (practical education)	31	32%
	HBO (applied science)	13	14%
	WO (university)	9	9%

Summaries of patient's family history

Symptom	*Number of responses*	*Number*	*Percentage*
Asthma	98	53	54%
Chronic bronchitis	98	46	47%
COPD	98	32	33%
Food allergy	98	26	27%
Allergy to inhaled material	98	39	40%
Allergy to animals	98	29	30%
Being 'always ill'	97	28	29%
Eczema	98	43	44%
Chronic sinusitis	98	30	31%
Chronic otitis	98	12	12%
Grommet placement	98	40	41%
Adenotonsillectomy	98	67	68%
Young people dying	98	38	39%
Deafness	98	38	39%
Autoimmune disease	98	25	26%
Rheumatoid arthritis	98	48	49%
Thyroid problems	98	33	34%
Coeliac disease	98	10	10%
Crohn's disease	98	8	8%
Ulcerative colitis	98	7	7%
Chronic intestinal inflammation	98	14	14%
Hay fever	98	46	47%
Cancer	98	68	69%
Leukemia	98	11	11%
Lymphoma	98	4	4%
Being 'always tired'	98	35	36%

Supplementary Table 2.2. Continued.

Combined family history*			
Symptoms	*Number of responses*	*Number*	*Percentage*
Pulmonary (COPD, asthma, bronchitis)	98	71	72%
Autoimmune (autoimmunity, rheumatoid arthritis, thyroid problems)	98	66	67%
IBD (Crohn's disease, ulcerative colitis, intestinal inflammation)	98	20	20%
Ear (sinusitis, otitis)	98	37	38%
Fatigue (always ill, always tired)	98	43	44%
Cancer (leukemia, lymphoma)	98	13	13%
Allergy (allergy to food, material, animals, eczema, hay fever)	97	70	71%
Pulmonary and/or ear	98	78	80%
Cancer, IBD and/or autoimmune	98	73	74%

Disease status			
Variable	*Category*	*Number*	*Percentage*
Patient always ill	Yes	87	89%
	No (same as other people)	8	8%
	No (less frequently than others)	3	3%
Most important complaint	Airway infection	35	36%
	Chronic cough	4	4%
	ENT infection	9	9%
	Tired, no energy	35	36%
	Other	15	15%

Supplementary Table 2.2. Continued.

Summaries of previous symptoms (yes/no binary variables)			
Symptom	*Number of responses*	*Number*	*Percentage*
Problems with vision	98	38	39%
Problems with hearing	98	30	31%
Otitis in the past	98	33	34%
Sinusitis in the past	98	51	52%
Grommets placed (past)	98	10	10%
Adenotonsillectomy (past)	98	53	54%
Otitis nowadays	97	10	10%
Sinusitis nowadays	98	41	42%
Surgery due to chronic sinusitis	98	17	17%
Dyspnoea (at rest)	98	35	36%
Dyspnoea (exercising)	98	61	62%
Dyspnoea (change in temperature)	98	47	48%
Dyspnoea (cigarette smoke)	98	52	53%
Dyspnoea (strong smells)	98	42	43%
Coughing productive	98	41	42%
Bladder infections	98	29	30%
Watery diarrhea	98	1	1%
Diarrhea (not watery)	98	12	12%
Eczema	98	25	26%
Other skin problems	98	42	43%
Pain in your bones/joints	98	70	71%
Red/swollen joints	98	24	24%
Chest pain during exercise	98	19	19%
Summaries of previous symptoms (variables with multiple categories)			
Suffer coughing	Never	3	3%
	Sometimes	51	52%
	Daily <8 weeks	17	17%
	Chronic >8 weeks	27	28%
Season of most complaints	Spring	16	17%
	Summer	14	15%
	Autumn	14	15%
	Winter	52	54%
How is your appetite	Bad	5	5%
	All Right	30	31%
	Good	63	64%

Supplementary Table 2.2. Continued.

Summaries of TAAQOOL domain scores			
TAAQOL domain	*Number of responses*	*Median*	*Inter-quartile range*
Gross motor functioning	98	63	25, 88
Fine Motor functioning	98	100	75, 100
Cognition	98	63	31, 100
Sleep	98	56	31, 81
Pain	98	50	36, 75
Social contacts	98	81	63, 100
Daily activities	97	50	22, 88
Sex	97	88	50, 100
Vitality	98	29	8, 50
Happiness	98	50	33, 67
Depressive mood	98	75	50, 83
Anger	97	89	67, 100

Summaries of original TAAQOL questions (Q1-30, part 1)				
Question	*No*	*A little*	*Some*	*A lot*
1. Difficulty walking up stairs	37 (38%)	24 (24%)	17 (17%)	20 (20%)
2. Difficulty bending over	46 (47%)	20 (20%)	18 (18%)	14 (14%)
3. Difficulty walking 500 yards	48 (49%)	18 (18%)	21 (21%)	11 (11%)
4. Difficulty lifting	41 (42%)	23 (23%)	19 (19%)	15 (15%)
5. Difficulty with scissors	83 (84%)	6 (6%)	3 (13%)	6 (6%)
6. Difficulty fastening buttons	77 (79%)	13 (13%)	2 (2%)	6 (6%)
7. Difficulty opening a can	71 (72%)	11 (11%)	11 (11%)	5 (5%)
8. Difficulty twisting jar lid	63 (64%)	14 (14%)	14 (14%)	7 (7%)
9. Difficulty concentrating on others	51 (52%)	23 (23%)	11 (11%)	13 (13%)
10. Difficulty remembering things	40 (41%)	28 (14%)	14 (14%)	16 (16%)
11. Difficulty concentrated thinking	42 (43%)	26 (27%)	12 (12%)	18 (18%)
12. Mind wandered	40 (41%)	26 (27%)	11 (11%)	21 (21%)
Question	*Never*	*Occasionally*	*Often*	*Always*
13. Difficulty getting to sleep	32 (33%)	46 (47%)	10 (10%)	10 (10%)
14. Slept restlessly	26 (27%)	38 (39%)	23 (23%)	11 (11%)
15. Lay awake a lot at night	34 (35%)	38 (39%)	17 (17%)	9 (9%)
16. Good night's sleep	11 (11%)	31 (32%)	25 (26%)	31 (32%)
17. Back-ache	35 (36%)	31 (32%)	17 (17%)	15 (15%)
18. Pain in neck/shoulders	24 (24%)	28 (29%)	28 (29%)	18 (18%)
19. Pain in joints/limbs	29 (30%)	27 (28%)	29 (30%)	13 (13%)
20. Pain in muscles	33 (34%)	30 (31%)	21 (21%)	14 (14%)
Question	*Often*	*Occasionally*	*Seldom*	*Never*
21. Talk others in confidence	63 (64%)	23 (23%)	6 (6%)	6 (6%)
22. Nice time with other people	55 (56%)	26 (27%)	11 (11%)	6 (6%)
23. Visit friends	44 (45%)	33 (34%)	15 (15%)	6 (6%)
24. Have good time with others	54 (55%)	30 (31%)	9 (9%)	5 (5%)

Supplementary Table 2.2. Continued.

Question	*No*	*A little*	*Some*	*A lot*
25. Difficulty with work/study	30 (31%)	17 (18%)	28 (29%)	22 (23%)
26. Done less work/study	39 (41%)	18 (19%)	18 (19%)	21 (22%)
27. Problems with types work	31 (32%)	21 (22%)	26 (27%)	19 (20%)
28. Worked less conscientiously	47 (48%)	19 (20%)	19 (20%)	12 (12%)
29. Less sex	49 (51%)	14 (14%)	16 (16%)	18 (19%)
30. Found sex less satisfying	64 (66%)	11 (11%)	13 (13%)	9 (9%)
Summaries of original TAAQOL questions (Q1-30, part 2)				
Question	*Not at all*	*A little*	*Quite a lot*	*Very much*
1. Difficulty walking up stairs	3 (5%)	20 (33%)	19 (31%)	19 (31%)
2. Difficulty bending over	3 (6%)	15 (29%)	17 (33%)	17 (33%)
3. Difficulty walking 500 yards	3 (6%)	14 (28%)	8 (16%)	25 (50%)
4. Difficulty lifting	4 (7%)	19 (33%)	15 (26%)	19 (33%)
5. Difficulty with scissors	3 (20%)	6 (40%)	4 (27%)	2 (13%)
6. Difficulty fastening buttons	3 (14%)	11 (52%)	5 (24%)	2 (10%)
7. Difficulty opening a can	3 (11%)	9 (33%)	9 (33%)	6 (22%)
8. Difficulty twisting jar lid	3 (9%)	13 (37%)	11 (31%)	8 (23%)
9. Difficulty concentrating on others	4 (9%)	14 (30%)	18 (38%)	11 (23%)
10. Difficulty remembering things	5 (9%)	15 (26%)	22 (38%)	16 (28%)
11. Difficulty concentrated thinking	3 (5%)	20 (36%)	15 (27%)	18 (32%)
12. Mind wandered	11 (19%)	17 (29%)	20 (34%)	10 (17%)
13. Difficulty getting to sleep	11 (17%)	32 (48%)	11 (17%)	12 (18%)
14. Slept restlessly	6 (8%)	36 (50%)	18 (25%)	12 (17%)
15. Lay awake a lot at night	5 (8%)	28 (44%)	18 (28%)	13 (20%)
16. Good night's sleep	23 (35%)	18 (27%)	13 (20%)	12 (18%)
17. Back-ache	4 (6%)	25 (40%)	15 (24%)	19 (30%)
18. Pain in neck/shoulders	5 (7%)	29 (39%)	21 (28%)	19 (26%)
19. Pain in joints/limbs	4 (6%)	25 (36%)	27 (39%)	13 (19%)
20. Pain in muscles	9 (14%)	26 (40%)	19 (29%)	11 (17%)
21. Talk others in confidence	14 (41%)	15 (44%)	3 (9%)	2 (6%)
22. Nice time with other people	7 (17%)	12 (29%)	10 (24%)	13 (31%)
23. Visit friends	16 (30%)	13 (25%)	16 (30%)	8 (15%)
24. Have good time with others	19 (44%)	10 (24%)	12 (28%)	2 (5%)
25. Difficulty with work/study	1 (1%)	14 (21%)	23 (34%)	29 (43%)
26. Done less work/study	2 (4%)	17 (30%)	19 (33%)	19 (33%)
27. Problems with types work	0 (0%)	22 (33%)	17 (26%)	27 (41%)
28. Worked less conscientiously	4 (8%)	16 (32%)	12 (24%)	18 (36%)
29. Less sex	10 (21%)	20 (42%)	12 (25%)	6 (13%)
30. Found sex less satisfying	7 (21%)	11 (33%)	8 (24%)	7 (21%)

Supplementary Table 2.2. Continued.

Summaries of original TAAQOL questions (Q31-45)				
Question	*No*	*A little*	*Quite*	*Very*
31. Energetic	45 (46%)	28 (29%)	20 (20%)	5 (5%)
32. Tired	10 (10%)	20 (20%)	34 (35%)	34 (35%)
33. Fit	48 (49%)	29 (30%)	16 (16%)	5 (5%)
34. Exhausted quickly	15 (15%)	19 (19%)	30 (31%)	34 (35%)
35. Joyful	13 (13%)	36 (37%)	35 (36%)	14 (14%)
36. Sad	35 (36%)	41 (42%)	17 (17%)	5 (5%)
37. In good spirits	19 (20%)	30 (13%)	37 (38%)	11 (11%)
38. Angry	44 (45%)	34 (35%)	11 (11%)	9 (9%)
39. Worried	19 (19%)	29 (30%)	31 (32%)	19 (19%)
40. Gloomy	44 (45%)	35 (36%)	10 (10%)	9 (9%)
41. Aggressive	71 (72%)	19 (19%)	5 (5%)	3 (3%)
42. Happy	6 (6%)	31 (32%)	40 (41%)	21 (21%)
43. Short-tempered	69 (71%)	22 (23%)	4 (4%)	2 (2%)
44. Cheerful	11 (11%)	32 (33%)	46 (47%)	9 (9%)
45. Anxious	49 (50%)	28 (29%)	13 (13%)	8 (8%)

Summaries of normally distributed laboratory test results			
Variable	*Number of responses*	*Mean*	*Standard deviation*
Leukocyte count (10^9/l)	98	7.6	2.8
Thrombocyte count (10^9/l)	98	270	73
Lymphocyte count (10^9/l)	98	2.1	0.7
Monocyte count (10^9/l)	98	0.42	0.14
T-helper lymphocytes (10^9/l)	84	0.94	0.40
Classical complement pathway activity (%)	80	109	24
Alternative complement pathway activity (%)	80	100	25

Supplementary Table 2.2. Continued.

Summaries of non-normally distributed laboratory test results			
Variable	*Number of responses*	*Median*	*Inter-quartile range*
Granulocyte count (10^9/l)	98	4.2	3.2, 5.9
Eosinophil count (10^9/l)	98	0.1	0.1, 0.2
Basophil count (10^9/l)	98	0.0	0.0, 0.1
Erythrocyte sedimentation rate (mm/1st hour)	74	4	2, 12
CRP (mg/l)	71	3	3, 6
T lymphocytes (10^9/l)	84	1.40	1.12, 1.90
Cytotoxic T lymphocytes (10^9/L)	84	0.48	0.31, 0.68
Natural killer cells (10^9/l)	83	0.22	0.15, 0.34
B lymphocytes (10^9/l)	84	0.20	0.11, 0.30
Total memory B lymphocytes (10^9/l)	74	0.05	0.03, 0.08
Non-switched memory B lymphocytes (10^9/l)	74	0.003	0.001, 0.007
Switched memory B lymphocytes (10^9/l)	74	0.019	0.009, 0.036
CD21 low B lymphocytes (10^9/l)	74	0.006	0.003, 0.010
Naive mature B lymphocytes (10^9/l)	74	0.09	0.05, 0.16
Transitional B lymphocytes (10^9/l)	74	0.008	0.003, 0.015
Plasmablasts (10^9/l)	74	0.0013	0.0005, 0.0037
Total IgG (g/l)	99	7.6	6.1, 9.6
IgA (g/l)	99	1.4	0.7, 2.3
IgM (g/l)	99	0.6	0.4, 1.0
IgG1 subclass (g/l)	96	5.1	4.0, 6.2
IgG2 subclass (g/l)	96	1.5	1.2, 2.3
IgG3 subclass (g/l)	96	0.31	0.21, 0.43
IgG4 subclass (g/l)	96	0.17	0.07, 0.39
Total IgE (IU/ml)	86	23	7, 72
Diphtheria titer before vaccination (IU/ml)	86	0.05	0.02, 0.12
Diphtheria titer after vaccination (IU/ml)	80	0.57	0.24, 1.43
Tetanus titer before vaccination (IU/ml)	86	0.74	0.17, 1.49
Tetanus titer after vaccination (IU/ml)	80	8.7	2.8, 16.0

Summaries of values below, within or above age-related reference ranges				
Parameter	*Normal range*	*Below*	*In Range*	*Above*
Lymphocyte (10^9/l)	1.1 - 2.5	8 (8%)	70 (71%)	20 (20%)
IgG (g/l)	7.0 - 16.0	41 (41%)	53 (54%)	5 (5%)
IgA (g/l)	0.7 - 4.0	24 (24%)	69 (70%)	6 (6%)
IgM (g/l)	0.4 - 2.3	33 (33%)	64 (65%)	2 (2%)
IgG1 (g/l)	4.9 – 11.4	44 (46%)	50 (52%)	2 (2%)
IgG2 (g/l)	1.5 – 6.4	51 (53%)	44 (46%)	1 (1%)
IgG3 (g/l)	0.2 – 1.1	20 (21%)	74 (77%)	2 (2%)
IgG4 (g/l)	0.08 – 1.4	27 (28%)	68 (71%)	1 (1%)

Supplementary Table 2.2. Continued.

Summaries of pneumococcal serotypes			
Before vaccination	*Number of responses*	*Median*	*Inter-quartile range*
Serotype 1 (microg/ml)	43	0.24	0.13, 0.68
Serotype 3 (microg/ml)	43	0.26	0.08, 0.60
Serotype 4 (microg/ml)	43	0.12	0.04, 0.27
Serotype 5 (microg/ml)	42	0.13	0.04, 0.47
Serotype 6 (microg/ml)	88	0.13	0.04, 0.37
Serotype 7f (microg/ml)	43	0.33	0.08, 0.78
Serotype 8 (microg/ml)	45	0.21	0.12, 0.41
Serotype 9v (microg/ml)	89	0.11	0.04, 0.27
Serotype 14 (microg/ml)	88	0.40	0.10, 1.95
Serotype 15b (microg/ml)	45	0.12	0.00, 0.39
Serotype 18c (microg/ml)	44	0.52	0.11, 0.91
Serotype 19f (microg/ml)	89	0.27	0.08, 1.75
Serotype 20 (microg/ml)	45	0.33	0.13, 1.25
Serotype 23f (microg/ml)	88	0.15	0.04, 0.80
Serotype 33f (microg/ml)	45	0.28	0.08, 0.66
After vaccination	*Number of responses*	*Median*	*Inter-quartile range*
Serotype 1 (microg/ml)	44	1.4	0.3, 4.3
Serotype 3 (microg/ml)	43	0.9	0.4, 3.8
Serotype 4 (microg/ml)	43	0.4	0.1, 1.5
Serotype 5 (microg/ml)	43	1.7	0.2, 6.7
Serotype 6 (microg/ml)	85	0.7	0.2, 5.2
Serotype 7f (microg/ml)	44	1.5	0.20, 7.1
Serotype 8 (microg/ml)	42	2.9	1.0, 13.0
Serotype 9v (microg/ml)	86	0.6	0.2, 2.7
Serotype 14 (microg/ml)	86	3.3	0.5, 16.3
Serotype 15b (microg/ml)	43	1.8	0.4, 8.7
Serotype 18c (microg/ml)	43	1.9	0.5, 6.7
Serotype 19f (microg/ml)	86	0.9	0.2, 4.0
Serotype 20 (microg/ml)	43	1.1	0.5, 10.0
Serotype 23f (microg/ml)	86	0.6	0.1, 4.8
Serotype 33f (microg/ml)	43	1.6	0.5, 7.3

Response to diphtheria, tetanus and pneumococcal vaccination		
Protein vaccine	*Criteria*	*number/total determined (percentage)*
Diphtheria	≥ fourfold increase and above 0.1 (IU/ml)	50/77 (65%)
	≥ fourfold increase and above 1.0 (IU/ml)	22/77 (29%)
Tetanus	≥ fourfold increase and above 0.1 (IU/ml)	58/80 (73%)
	≥ fourfold increase and above 1.0 (IU/ml)	55/80 (69%)
Pneumococcal polysaccharide vaccine	*Before >0.35 (microg/ml)*	*After >1.0 (microg/ml)*

Supplementary Table 2.2. Continued.

Serotype 1	17/43 (40%)	20/44 (55%)
Serotype 3	16/43 (37%)	21/43 (49%)
Serotype 4	8/43 (19%)	16/43 (37%)
Serotype 5	12/42 (29%)	24/43 (56%)
Serotype 6	23/88 (26%)	37/85 (44%)
Serotype 7f	21/43 (49%)	25/44 (57%)
Serotype 8	11/45 (24%)	30/42 (71%)
Serotype 9v	21/89 (24%)	35/86 (41%)
Serotype 14	45/88 (51%)	58/86 (67%)
Serotype 15b	16/45 (36%)	25/43 (58%)
Serotype 18c	24/44 (55%)	26/43 (60%)
Serotype 19f	41/89 (46%)	41/86 (48%)
Serotype 20	21/45 (47%)	22/43 (51%)
Serotype 23f	32/88 (36%)	38/86 (44%)
Serotype 33f	20/45 (44%)	24/43 (56%)

The number of serotypes where the after measurements are >1.0 were calculated for each patient; 59 of 86 patients (69%) measured have <7 values >1.0, and are classified as abnormal (laboratory reference values).

Summaries of categorical laboratory test results			
Variable	*Category*	*Number*	*Percentage*
M protein	Not present	68	99%
	Present – not monoclonal	1	1%
	Present - monoclonal	0	0%
ANA	Negative	75	85%
	Positive	14	15%
Rheumatic factor	Negative	30	88%
	Positive	4	12%
IgE for tree pollen	Class 0	58	84%
	Class 1 - 3	6	9%
	Class 4 - 6	5	7%
IgE for house dust mite	Class 0	59	86%
	Class 1 - 3	8	12%
	Class 4 - 6	2	3%
IgE for cat dander	Class 0	62	90%
	Class 1 - 3	4	6%
	Class 4 - 6	3	4%
IgE for dog dander	Class 0	62	90%
	Class 1 - 3	7	10%
	Class 4 - 6	0	0%
IgE for grass pollen	Class 0	57	83%
	Class 1 - 3	9	13%
	Class 4 - 6	3	4%

Supplementary Table 2.2. Continued.

Aspergillus IgG	Negative	16	73%
	Positive	6	27%

*Supplementary Table 2.2. *The original family history questions were combined to give a more condensed family history. A family history in each of the categories was defined as one or more of the conditions being present. Abbreviations: ANA = antinuclear antibody, BMI = body mass index, COPD = chronic obstructive pulmonary disease, CRP = C-reactive protein, CVID = common variable immunodeficiency disorders, ENT = ear-nose-throat, HRCT = high resolution computed tomography, IBD = inflammatory bowel disease, Ig = immunoglobulin, IVIG = intravenous immunoglobulin substitution, Q = question, SCIG = subcutaneous immunoglobulin substitution, TAAQOL = TNO-AZL Questionnaire for Adult Health-Related Quality of Life [https://www.tno.nl/media/4727/vragenlijsten_01032012.pdf; accessed June 2017].*

Supplementary table 2.3. Statistical analyses in 99 adults with primary antibody deficiency.

Comparisons between referring doctor types						
Variable	*Category*	*GP*	*Internist*	*Pulmonologist*	*Other*	*p-value*
Diagnosis	CVID	3 (9%)	3 (20%)	3 (7%)	1 (17%)	0.06
	unPAD	32 (91%)	12 (80%)	40 (93%)	5 (83%)	
HRCT bronchial wall thickening	No	9 (50%)	5 (56%)	16 (52%)	1 (50%)	1.00
	Yes	9 (50%)	4 (44%)	15 (48%)	1 (50%)	
HRCT bronchiectasis	0/1 lobes	14 (78%)	8 (89%)	16 (52%)	2 (100%)	0.08
	2+ lobes	4 (22%)	1 (11%)	15 (48%)	0 (0%)	
HRCT mucus plugging	No	16 (89%)	8 (89%)	24 (77%)	1 (50%)	0.41
	Yes	2 (11%)	1 (11%)	7 (23%)	1 (50%)	
HRCT atelectasis	No	15 (83%)	9 (100%)	24 (77%)	2 (100%)	0.50
	Yes	3 (17%)	0 (0%)	7 (23%)	0 (0%)	

Comparisons between patient diagnoses				
Variable	*Category*	*CVID*	*unPAD*	*p-value*
HRCT bronchial wall thickening	No	3 (43%)	28 (53%)	0.62
	Yes	4 (57%)	25 (47%)	
HRCT bronchiectasis	0/1 lobes	6 (86%)	34 (64%)	0.26
	2+ lobes	1 (14%)	19 (36%)	
HRCT mucus plugging	No	7 (100%)	42 (79%)	0.18
	Yes	0 (0%)	11 (21%)	
HRCT atelectasis	No	7 (100%)	43 (81%)	0.21
	Yes	0 (0%)	10 (19%)	
Therapy (related to antibody deficiency diagnosis)	None	0 (0%)	50 (56%)	**<0.001**
	Antibiotic prophylaxis	1 (10%)	19 (21%)	
	IVIG or SCIG	9 (90%)	20 (22%)	

Supplementary table 2.3. Continued.

Differences between patients receiving different therapies					
Variable	*Category*	*None*	*Antibiotic prophylaxis*	*IVIG or SCIG*	*p-value*
HRCT bronchial wall thickening	No	10 (42%)	8 (57%)	13 (59%)	0.51
	Yes	14 (58%)	6 (43%)	9 (41%)	
HRCT bronchiectasis	0/1 lobes	16 (67%)	7 (50%)	17 (77%)	0.27
	2+ lobes	8 (33%)	7 (50%)	5 (23%)	
HRCT mucus plugging	No	20 (83%)	10 (71%)	19 (86%)	0.58
	Yes	4 (17%)	4 (29%)	3 (14%)	
HRCT atelectasis	No	19 (79%)	11 (79%)	20 (91%)	0.50
	Yes	5 (21%)	3 (21%)	2 (9%)	

Comparison of genders				
Variable	*Category*	*Males*	*Females*	*p-value*
Diagnosis	CVID	5 (18%)	5 (7%)	0.17
	UnPAD	23 (82%)	66 (93%)	
HRCT bronchial wall thickening	No	9 (43%)	22 (51%)	1.00
	Yes	8 (47%)	21 (49%)	
HRCT bronchiectasis	0/1 lobes	10 (59%)	30 (70%)	0.55
	2+ lobes	7 (41%)	13 (30%)	
HRCT mucus plugging	No	13 (76%)	36 (84%)	0.71
	Yes	4 (24%)	7 (16%)	
HRCT atelectasis	No	13 (76%)	37 (86%)	0.45
	Yes	4 (24%)	6 (14%)	
Therapy (related to antibody deficiency diagnosis)	None	11 (39%)	39 (55%)	0.37
	Antibiotic prophylaxis	7 (25%)	13 (19%)	
	IVIG or SCIG	10 (36%)	19 (27%)	

Differences between most important complaint categories				
Variable	*Category*	*ENT/airway/cough*	*Tired/other*	*p-value*
Always ill	No	4 (8%)	7 (14%)	0.53
	Yes	44 (92%)	43 (86%)	
HRCT bronchial wall thickening	No	16 (50%)	14 (52%)	1.00
	Yes	16 (50%)	3 (48%)	
HRCT bronchiectasis	0/1 lobes	20 (62%)	19 (70%)	0.59
	2+ lobes	12 (38%)	8 (30%)	
HRCT mucus plugging	No	23 (72%)	25 (93%)	**0.05**
	Yes	9 (28%)	2 (7%)	
HRCT atelectasis	No	26 (81%)	23 (85%)	0.74
	Yes	6 (19%)	4 (15%)	
Gender	Male	12 (25%)	16 (32%)	0.51
	Female	36 (75%)	34 (68%)	

Supplementary table 2.3. Continued.

Associations with being always ill				
Variable	*Category*	*Not always ill*	*Always ill*	*p-value*
HRCT bronchial wall thickening	No	1 (20%)	29 (54%)	0.20
	Yes	4 (80%)	25 (46%)	
HRCT bronchiectasis	0/1 lobes	4 (80%)	35 (65%)	0.65
	2+ lobes	1 (20%)	19 (35%)	
HRCT mucus plugging	No	5 (100%)	43 (80%)	0.57
	Yes	0 (0%)	11 (20%)	
HRCT atelectasis	No	5 (100%)	44 (81%)	0.58
	Yes	0 (0%)	10 (19%)	
Gender	Male	4 (36%)	24 (28%)	0.51
	Female	7 (64%)	63 (72%)	

Associations with presence of ≥1 of red/swollen joints and/or pain in bones/joints				
Variable	*Category*	*Absence*	*Presence*	*p-value*
Always ill	No	4 (15%)	7 (10%)	0.49
	Yes	23 (85%)	64 (90%)	
HRCT bronchial wall thickening	No	8 (53%)	22 (50%)	1.00
	Yes	7 (47%)	22 (50%)	
HRCT bronchiectasis	0/1 lobes	9 (60%)	30 (68%)	0.75
	2+ lobes	6 (40%)	14 (32%)	
HRCT mucus plugging	No	10 (67%)	38 (86%)	0.13
	Yes	5 (33%)	6 (14%)	
HRCT atelectasis	No	11 (87%)	38 (86%)	0.25
	Yes	4 (13%)	6 (14%)	
Most important complaint	Ear/airway/cough	17 (63%)	31 (44%)	0.11
	Tired/other	10 (37%)	40 (56%)	
Gender	Male	11 (41%)	17 (24%)	0.13
	Female	16 (59%)	54 (76%)	

Associations with ≥1 of coughing productive, surgery chronic sinusitis, sinusitis nowadays, otitis nowadays, sinusitis in the past, otitis in the past				
Variable	*Category*	*Absence*	*Presence*	*p-value*
Always ill	No	5 (36%)	6 (7%)	**0.008**
	Yes	9 (64%)	78 (93%)	
HRCT bronchial wall thickening	No	4 (40%)	26 (53%)	0.51
	Yes	6 (60%)	23 (47%)	
HRCT bronchiectasis	0/1 lobes	7 (70%)	32 (65%)	1.00
	2+ lobes	3 (30%)	17 (35%)	
HRCT mucus plugging	No	8 (80%)	40 (82%)	1.00
	Yes	2 (20%)	9 (18%)	

Supplementary table 2.3. Continued.

HRCT atelectasis	No	9 (90%)	40 (82%)	1.00
	Yes	1 (10%)	9 (18%)	
Most important complaint	Ear/airway/cough	5 (%)	43 (51%)	0.39
	Tired/other	9 (64%)	41 (49%)	
Gender	Male	3 (21%)	25 (30%)	0.75
	Female	11 (79%)	59 (70%)	

Associations with season of the year with most complaints

Variable	*Category*	*Spring - summer*	*Autumn - winter*	*p-value*
Family history of allergy	No	8 (27%)	18 (27%)	1.00
	Yes	22 (73%)	48 (73%)	
HRCT bronchial wall thickening	No	7 (44%)	21 (51%)	0.77
	Yes	9 (56%)	20 (49%)	
HRCT bronchiectasis	0/1 lobes	11 (69%)	28 (68%)	1.00
	2+ lobes	5 (31%)	13 (32%)	
HRCT mucus plugging	No	12 (75%)	34 (83%)	0.48
	Yes	4 (25%)	7 (17%)	
HRCT atelectasis	No	13 (81%)	35 (85%)	0.70
	Yes	3 (19%)	6 (15%)	

Comparison of TAAQOL domains between Dutch reference population and Care Path data

Domain	*Reference data*	*Care Path data*	*p-value*
Gross motor functioning	100 [81, 100]	63 [25, 88]	**<0.001**
Fine motoric functioning	100 [100, 100]	100 [75, 100]	**<0.001**
Cognitive functioning	93 [75, 100]	63 [31, 100]	**<0.001**
Sleep	81 [56, 94]	56 [31, 81]	**<0.001**
Pain	81 [63, 94]	50 [38, 75]	**<0.001**
Social functioning	88 [75, 100]	81 [63, 100]	**0.002**
Daily activities	100 [75, 100]	50 [25, 88]	**<0.001**
Sexuality	100 [75, 100]	88 [50, 100]	**0.002**
Vitality	67 [50, 83]	29 [8, 50]	**<0.001**
Positive emotions	67 [58, 75]	50 [33, 67]	**<0.001**
Depressive emotions	83 [67, 92]	75 [50, 83]	**<0.001**
Aggressive emotions	89 [78, 100]	89 [67, 100]	0.01

Associations with ANA positivity

Variable	*Category*	*ANA negative*	*ANA positive*	*p-value*
Bone / joint pain	No	20 (27%)	3 (23%)	1.00
	Yes	55 (73%)	10 (77%)	
Fine motor skills	-	100 [75, 100]	100 [75, 100]	0.97
Family history of thyroid disease	No	48 (64%)	8 (62%)	1.00
	Yes	27 (36%)	5 (39%)	
Age	-	52.1 ± 15.6	48.2 ± 16.4	0.40

Supplementary table 2.3. Continued.

Gender	Male	21 (28%)	4 (29%)	1.00
	Female	54 (72%)	10 (71%)	
Associations with Rheumatoid factor positivity				
Variable	*Category*	*RF negative*	*RF positive*	*p-value*
ANA	Negative	26 (87%)	3 (75%)	0.49
	Positive	4 (13%)	1 (25%)	
Bone / joint pain	No	1 (3%)	1 (25%)	0.23
	Yes	29 (97%)	4 (75%)	
Fine motor skills	-	88 [50, 100]	78 [53, 91]	0.56
Family history of thyroid disease	No	19 (63%)	3 (75%)	1.00
	Yes	11 (37%)	1 (25%)	
Age	-	56.6 ± 14.5	67.0 ± 6.9	0.17
Gender	Male	6 (25%)	1 (25%)	1.00
	Female	24 (80%)	3 (75%)	
Associations with IgE Class 4-6 (IgE positive)				
ANA	Negative	51 (85%)	4 (57%)	0.10
	Positive	9 (15%)	3 (43%)	
Rheumatoid factor	Negative	23 (96%)	1 (100%)	1.00
	Positive	1 (4%)	0 (0%)	
Bone / joint pain	No	18 (30%)	2 (25%)	1.00
	Yes	42 (70%)	6 (75%)	
Fine motor skills	-	100 [84, 100]	100 [94, 100]	0.38
Family history of thyroid disease	No	38 (63%)	6 (75%)	0.70
	Yes	22 (37%)	2 (25%)	
Age	-	50.4 ± 15.3	42.4 ± 15.8	0.17
Gender	Male	16 (26%)	3 (37%)	0.66
	Female	45 (74%)	5 (63%)	

Supplementary Table 2.3. Abbreviations: ANA = antinuclear antibody, CVID = common variable immunodeficiency disorders, GP = general practitioner, HRCT = high resolution computed tomography, Ig = immunoglobulin, IVIG = intravenous immunoglobulin, RF = rheumatoid factor, SCIG = subcutaneous immunoglobulin, TAAQOL = TNO-AZL Questionnaire for Adult Health-Related Quality of Life [https://www.tno.nl/media/4727/vragenlijsten_01032012.pdf; accessed June 2017], unPAD = unclassified antibody deficiency according to European Society for Immunodeficiencies (ESID) Registry criteria [https://esid.org/Working-Parties/Registry/Diagnosis-criteria; accessed June 2017]. P-values in bold are considered significant (< 0.01).

Supplementary table 2.4. Clustering analyses in 99 adults with primary antibody deficiency.

Analyses using the K-means clustering method with the variables age, female gender, smoking packyears, HRCT - bronchial wall thickening / 2+ lobes bronchiectasis / mucus plugging / atelectasis, TAAQOL domain scores, IgG, IgA, IgM, IgG1, IgG2, IgG3, IgG4, postvaccination titres to diphtheria / tetanus, number of pneumococcal serotypes <1.00 microg/ml postvaccination in the data analysis. Only patients with complete information on all variables could be included, and thus the analysis was based on 51 patients.

Number of clusters	*Calinski/Harabasz pseudo-F index*
2	**24.8**
3	15.7
4	11.2
5	10.8

Comparisons of variables among the patient groups in the two-cluster solution
{Figures are mean ± standard deviation, median [inter-quartile range] or number (percentage)}

Variable	*Cluster 1 (n=30)*	*Cluster 2 (n=21)*	*p-value*
Age	53.0 ± 14.7	55.8 ± 15.2	0.50
Female gender	23 (77%)	16 (76%)	1.00
Smoking packyears	6 [0, 20]	14 [5, 20	0.19
Bronchial wall	9 (30%)	6 (29%)	1.00
2+ lobes bronchiectasis	15 (50%)	13 (62%)	0.57
Mucus plugging	8 (27%)	7 (33%)	0.76
Atelectasis	1 (3%)	1 (5%)	1.00
Gross motor functioning	75 [50, 100]	31 [6, 44]	**<0.001**
Fine motoric functioning	100 [88, 100]	88 [63, 100]	0.21
Cognitive functioning	93 [75, 100]	31 [13, 50]	**<0.001**
Sleep	81 [69, 100]	31 [19, 50]	**<0.001**
Pain	63 [38, 88]	38 [19, 56]	**0.005**
Social functioning	94 [75, 100]	56 [31, 69]	**<0.001**
Daily activities	75 [63, 100]	25 [13, 44]	**<0.001**
Sexuality	100 [88, 100]	38 [25, 88]	**<0.001**
Vitality	42 [25, 67]	8 [0, 25]	**<0.001**
Positive emotions	67 [50, 75]	33 [16, 58]	**0.003**
Depressive emotions	79 [67, 92]	42 [33, 67]	**<0.001**
Aggressive emotions	100 [89, 100]	67 [56, 100]	**0.005**
IgG	7.7 [5.9, 9.6]	7.0 [5.9, 7.8]	0.39
IgA	1.3 [0.7, 2.8]	1.4 [0.7, 1.8]	0.80
IgM	0.7 [0.3, 1.2]	0.5 [0.4, 0.8]	0.25
IgG1	5.2 [3.9, 6.5]	4.9 [4.0, 5.9]	0.25
IgG2	1.5 [1.1, 2.5]	1.2 [0.9, 1.6]	0.14
IgG3	0.3 [0.2, 0.4]	0.3 [0.2, 0.5]	0.99
IgG4	0.14 [0.07, 0.44]	0.15 [0.07, 0.28]	0.97
Diphtheria (post)	0.6 [0.1, 1.3]	0.6 [0.4, 1.3]	0.52
Tetanus (post)	6.0 [1.9, 14.1]	9.9 [3.9, 16.0]	0.33
Number pneumococcal serotypes <1 (post)	4.2 ± 2.9	4.8 ± 2.6	0.49
CVID diagnosis	5 (17%)	1 (5%)	0.38
unPAD diagnosis	25 (83%)	20 (95%)	

Supplementary table 2.4. Continued.

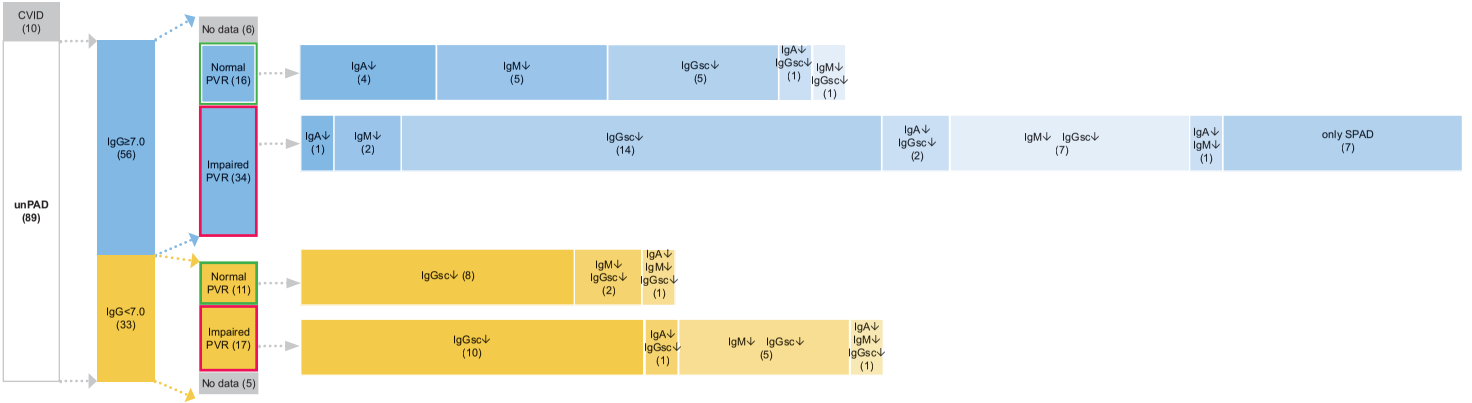
Analyses using the K-means clustering method with the same variables but omitting the HRCT-related variables in the data analysis, which had the most missing data. This analysis was based on 75 patients.

Number of clusters	*Calinski/Harabasz pseudo-F index*
2	**36.7**
3	24.9
4	20.6
5	16.9

Comparisons without HRCT-related variables among the patient groups in the two-cluster solution {Figures are mean ± standard deviation, median [inter-quartile range] or number (percentage)}

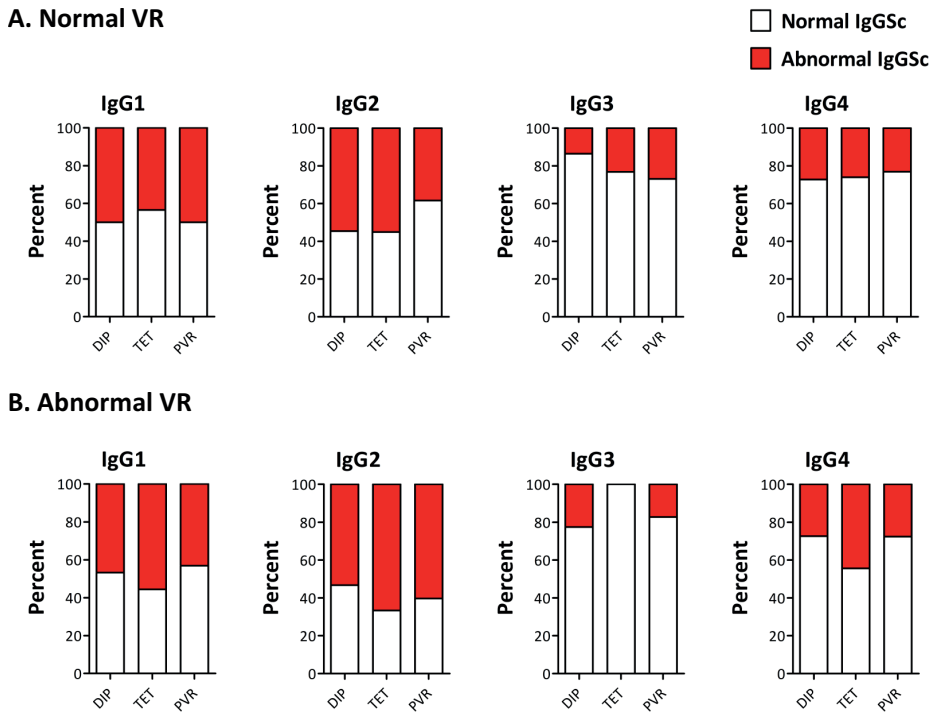
Variable	*Cluster 1 (n=38)*	*Cluster 2 (n=37)*	*p-value*
Age	50.9 ± 15.3	50.9 ± 16.1	0.99
Female gender	30 (79%)	28 (76%)	0.79
Smoking packyears	4 [0, 18]	8 [0, 19]	0.29
Gross motor functioning	84 [63, 100]	44 [13, 63]	**<0.001**
Fine motoric functioning	100 [88, 100]	88 [75, 100]	0.08
Cognitive functioning	97 [75, 100]	31 [19, 50]	**<0.001**
Sleep	75 [63, 100]	44 [25, 56]	**<0.001**
Pain	63 [50, 88]	38 [19, 56]	**<0.001**
Social functioning	100 [88, 100]	63 [44, 75]	**<0.001**
Daily activities	81 [63, 100]	25 [6, 38]	**<0.001**
Sexuality	100 [88, 100]	50 [25, 75]	**<0.001**
Vitality	46 [33, 75]	8 [0, 25]	**<0.001**
Positive emotions	67 [50, 75]	33 [25, 50]	**<0.001**
Depressive emotions	83 [67, 92]	58 [33, 67]	**<0.001**
Aggressive emotions	100 [89, 100]	77 [97, 89]	**<0.001**
IgG	7.4 [6.0, 9.5]	7.5 [6.2, 9.6]	0.56
IgA	1.2 [0.6, 2.2]	1.6 [1.1, 2.4]	0.22
IgM	0.8 [0.3, 1.4]	0.5 [0.4, 0.8]	0.16
IgG1	5.0 [3.9, 6.6]	5.1 [4.4, 6.5]	0.16
IgG2	1.4 [1.1, 2.2]	1.5 [1.2, 2.2]	0.66
IgG3	0.3 [0.2, 0.4]	0.4 [0.2, 0.5]	0.28
IgG4	0.13 [0.08, 0.25]	0.27 [0.07, 0.42]	0.14
Diphtheria (post)	0.7 [0.2, 1.5]	0.6 [0.3, 0.9]	0.89
Tetanus (post)	6.8 [2.2, 16.0]	9.9 [3.6, 16.0]	0.26
Number pneumococcal serotypes <1 (post)	4.6 ± 3.0	5.5 ± 2.6	0.14
CVID diagnosis	5 (13%)	1 (3%)	0.20
unPAD diagnosis	33 (87%)	36 (97%)	

Supplementary Table 2.4. Abbreviations: CVID = common variable immunodeficiency disorders, HRCT = high resolution computed tomography, Ig = immunoglobulin, TAAQOL = TNO-AZL Questionnaire for Adult Health-Related Quality of Life [https://www.tno.nl/media/4727/vragenlijsten_01032012.pdf; accessed June 2017], unPAD = unclassified antibody deficiency according to European Society for Immunodeficiencies (ESID) Registry criteria [https://esid.org/Working-Parties/Registry/Diagnosis-criteria; accessed June 2017]. P-values in bold are considered significant (<0.01).



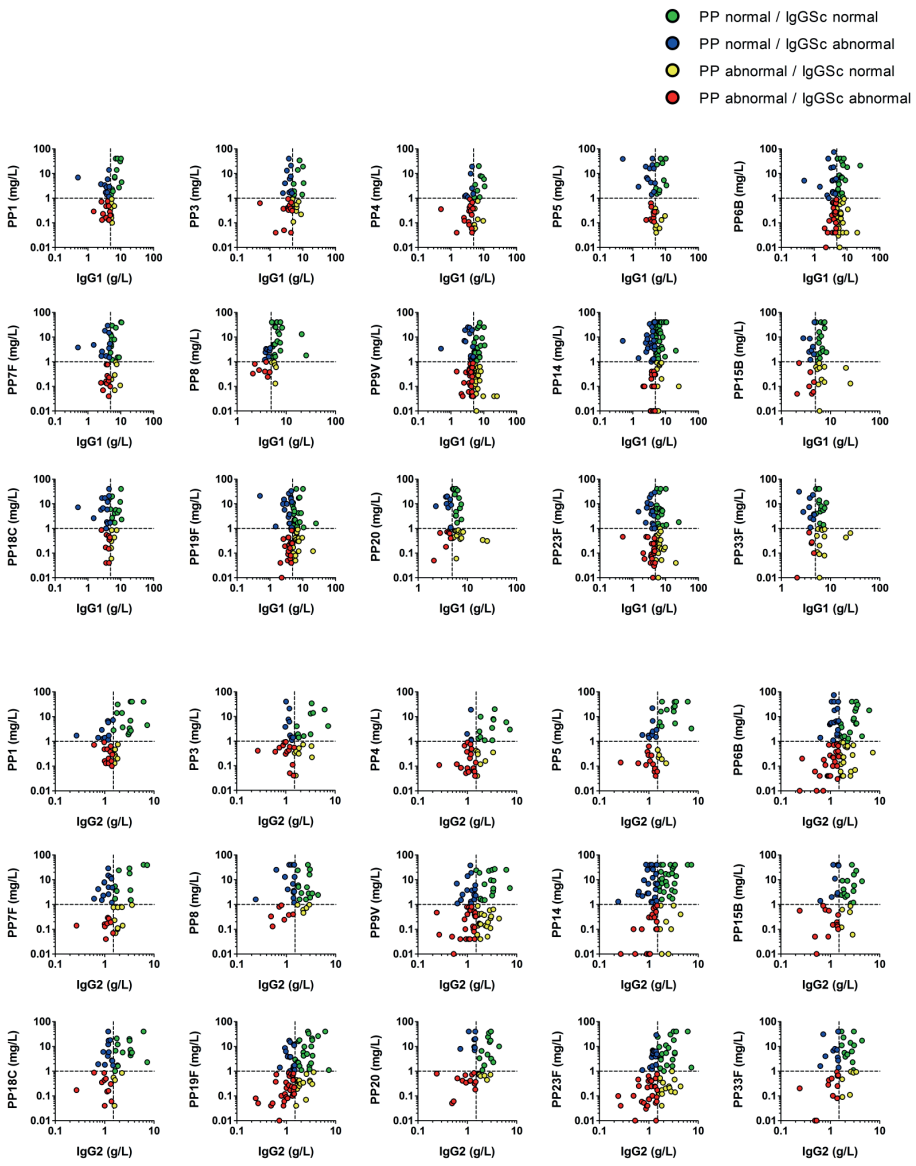
Supplementary Figure 2.1. Division into subgroups according to immunoglobulin levels and pneumococcal vaccination responses of the adult unPAD cohort.

Supplementary Figure 2.1. Abbreviations: CVID = common variable immunodeficiency disorder; IgGsc = IgG-subclass(es); PVR = pneumococcal vaccination response; SPAD = specific antibody deficiency; unPAD = unclassified primary antibody deficiency.



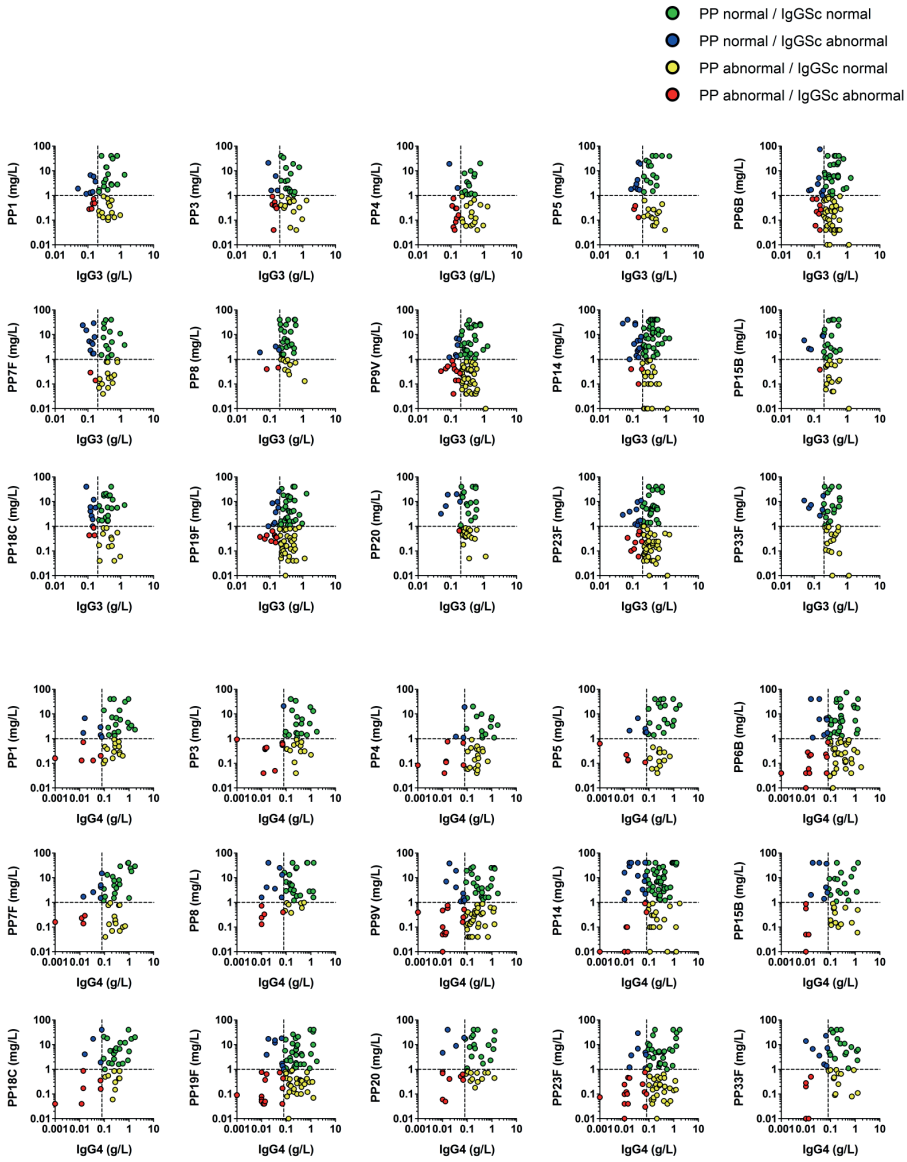
Supplementary Figure 2.2. Relation between vaccination responses and IgG-subclass levels.

Supplementary Figure 2.2. The bar graphs display the percentage of low IgG1, IgG2, IgG3 and IgG4 respectively between A. normal vaccination response, and B. abnormal vaccination response.



Supplementary Figure 2.3. Post-vaccination specific antibody titres against separate pneumococcal serotypes plotted against IgG1, IgG2 on a logarithmic scale.

Supplementary Figure 2.3. Per separate graph, patients were classified using the cut-off values for IgG-subclasses and vaccine responses to pneumococcal serotypes (dotted lines in the graphs). To be able to display patients' data points into the graphs, a pneumococcal serotype value of 0 g/L was changed to "0.01" g/L, which did not influence the classification.



Supplementary 2.4. Post-vaccination specific antibody titres against separate pneumococcal serotypes plotted against IgG3, and IgG4 on a logarithmic scale.

Supplementary Figure 2.4. Per separate graph, patients were classified using the cut-off values for IgG-subclasses and vaccine responses to pneumococcal serotypes (dotted lines in the graphs). To be able to display patients' data points into the graphs, a pneumococcal serotype value of 0 g/L was changed to "0.01" g/L, which did not influence the classification.

Chapter 3

Truly selective primary IgM deficiency is probably very rare

Lisanne MA Janssen, MD, Thomas Macken, MD, Marjonne CW Creemers, MD, PhD, Johannes FM Pruijt, MD, PhD, Jeroen JJ Eijk, MD, Esther de Vries, MD, PhD

Clin Exp Immunol. 2018; 191(2):203-211

ABSTRACT

Isolated decreased serum-IgM has been associated with severe and/or recurrent infections, atopy and autoimmunity. However, the reported high prevalence of clinical problems in IgM-deficient patients may reflect the skewed tertiary centre population studied so far. Also, many papers on IgM-deficiency have included patients with more abnormalities than just IgM-deficiency. We studied truly selective primary IgM deficiency according to the diagnostic criteria of the European Society for Immunodeficiencies ESID (*true sIgMdef)* by reviewing the literature (261 patients with primary decreased serum-IgM in 46 papers) and retrospectively analysing all patients with decreased serum-IgM in a large teaching hospital in 's-Hertogenbosch, the Netherlands (1-July-2005 to 23-March-2016; n=8,049 IgM<0.4g/l; n=2,064 solitary [IgG+IgA normal/IgM<age-matched reference]). 359/2064 (17%) cases from our cohort had primary isolated decreased serum-IgM, proven persistent in 45/359 (13%) cases; their medical charts were reviewed. Our main finding is that true sIgMdef is probably very rare. Only 6/261 (2%) literature cases and 3/45 (7%) cases from our cohort completely fulfilled the ESID criteria; 63/261 (24%) literature cases also had other immunological abnormalities and fulfilled the criteria for unclassified antibody deficiencies (*unPAD*) instead. The diagnosis was often uncertain (*possible sIgMdef*): data on IgG-subclasses and/or vaccination responses were lacking in 192/261 (74%) literature cases and 42/45 (93%) cases from our cohort. Our results also illustrate the clinical challenge of determining the relevance of a serum sample with decreased IgM; a larger cohort of true sIgMdef patients is needed to fully explore its clinical consequences. The ESID online Registry would be a good tool for this.

INTRODUCTION

IgM deficiency is on the one hand reported to be associated with a wide range of clinical presentations including severe or recurrent infections, atopy, autoimmunity and malignancy [1]. On the other hand, there are doubts about its clinical significance [2]; studies in healthy populations have shown that genetic polymorphisms as well as environmental factors may influence serum IgM levels [2,3]. Previous studies on the clinical significance of IgM deficiency have been affected by selection bias towards 'disease', as mostly symptomatic patients from tertiary centre cohorts have been described [4–6].

The European Society for Immunodeficiencies (ESID) Registry defines primary selective IgM deficiency (sIgMdef) as a serum IgM level repeatedly below 2 SDs of normal with normal levels of serum IgA, IgG and IgG-subclasses, normal vaccination responses, absence of T-cell defects and absence of causative external factors (http://www.esid.org). Many previously published articles that report on 'IgM deficiency' do not fulfil these criteria [7,8].

To facilitate a clear discussion, we define three categories in our study: (1) truly selective primary IgM deficiency (true sIgMdef) - the ESID criteria are *completely* fulfilled, which means serum IgM levels are repeatedly decreased and IgG, IgA, IgG-subclasses and vaccination responses have been determined and were normal for age; we consider the absence of *clinical* signs suggesting a T-cell defect sufficient; (2) possible selective primary IgM deficiency (possible sIgMdef) - the diagnosis of true sIgMdef is *uncertain*, which means that the ESID criteria are not completely fulfilled, because data on IgG-subclasses and/or vaccination responses are lacking; and (3) *unclassified* primary antibody deficiency (unPAD) - other abnormalities in antibodies are also present: IgG-subclass deficiency, below-normal levels of IgG or IgA, and/or impaired vaccination responses.

The aim of our study was to learn more about the clinical significance of true sIgMdef. Therefore, we first conducted a scoping review to identify all previously published patients with decreased serum IgM. Second, we analysed decreased serum IgM identified through the laboratory files of the Jeroen Bosch Hospital in 's-Hertogenbosch, the Netherlands, a large teaching hospital (secondary centre). Finally, we analysed whether these fulfilled the criteria for true sIgMdef.

MATERIALS AND METHOD

Literature search

The PubMed database was searched for articles concerning 'IgM deficiency' published until May 10, 2017 (no starting date). The search query was defined as {selective OR isolated} AND {IgM OR Immunoglobulin M} AND {deficiency OR low} AND {immunodeficiency syndromes}. We also screened the reference lists of articles identified by our search strategy and added those articles that reported about decreased serum IgM (snowball method). Our search strategy is described in detail in **Supplementary Figure 3.1**. We considered decreased serum IgM to be secondary in combination with the use of immunosuppressive agents, malignancy (e.g. clear cell sarcoma, promyelocytic leukaemia, multiple myeloma) or gastrointestinal loss (e.g. enteropathy through Crohn's or coeliac disease). Only papers that (also) contained patients with *primary* decreased serum IgM were included in the study. We analysed whether these patients fulfilled the criteria for *true* sIgMdef.

Our cohort

Patient selection

All serum immunoglobulin levels determined between July 1, 2005, and March 23, 2016, in the Jeroen Bosch Hospital (JBZ) in 's-Hertogenbosch, the Netherlands (encachment area 350,000; 500,000 outpatient visits & 32,000 admissions per year), were obtained (n=38,149; 5,342 (14%) samples from children and 32,509 (85%) samples from adults, missing age values in 298 samples). Of these, all samples with serum IgM values <0.4 g/l were selected (n=8,049, details in **Supplementary Figure 3.2**). Samples were excluded if serum IgM levels were normal according to age-matched reference values (these were all young children) [9]. To identify all *patients* with isolated decreased serum IgM, samples with decreased age-matched IgA- and/or IgG values as well as follow-up samples of serum-IgM were excluded. The medical charts were screened regarding patient history and medication use to exclude the samples from those patients in whom decreased serum IgM could be *secondary* (caused by external factors; definition see literature review above). Patients with cystic fibrosis (n=3) were excluded because their clinical symptoms would be difficult to interpret. Laboratory data of all primary cases were analysed to identify patients in whom serum IgM level was determined only once and in whom serum IgM level was repeatedly determined, but had normalized. Only the medical charts of patients with *persistent* decreased serum IgM levels were reviewed in detail; this patient group comprises both *possible* and *true* primary sIgMdef (definitions see Introduction). The Medical Ethical Committee Brabant approved the study.

Data collection

Data on demographics, clinical features, laboratory results and treatment, conclusions written by medical specialists and ICD-10-codes were derived from our electronic patient system. For clinical evaluation, we collected the type of medical specialist who discovered the decreased serum IgM, reason(s) for determining serum IgM, and clinical problems that could be related to antibody deficiency. We considered the following clinical problems to be possibly related to antibody deficiency: infections, atopic and/or autoimmune manifestations, inflammation of the gastrointestinal tract, long-lasting fatigue, depression and malignancies. Pneumonia required confirmation by thoracic X-ray. Allergic diseases (allergic rhino conjunctivitis, food allergy, allergic urticaria, allergic anaphylaxis) required confirmation by skin prick testing or RAST. For immunological evaluation, we collected data on serum IgM, IgG and IgA levels and - if determined - data on IgG subclasses, T-cell subsets and function, antibody responses to vaccinations, Isohemagglutinin levels, antinuclear antibodies (ANA) and specific serum IgE directed against inhalant allergens. For interpretation of serum immunoglobulins and lymphocyte subpopulations age-matched reference values were used [10]. Because our laboratory cut-off for serum IgM levels is 0.2 g/l, a value of < 0.2 g/l was replaced by 0.1 g/l for calculating mean serum IgM level (n = 4). For interpretation of pneumococcal antibody responses laboratory specific reference values were used [11]. The follow-up period was defined as the date of the first serum sample with decreased IgM until the date of data extraction. All patient data were encrypted and saved on a protected server using Research Manager software developed by Cloud9 Health Solutions (Deventer, the Netherlands).

Statistical analysis

Statistical analysis was performed using SPSS for Windows, version 21. Descriptive statistics were used to compute frequencies of categorical variables and mean (with SD) or median (with IQR) of continuous variables depending on the distribution.

RESULTS

Literature search

Supplementary Table 3.1 gives an overview of the identified relevant literature. 261 patients with primary decreased serum IgM were described in 46 papers. 8 patients (2 adults and 6 children) fulfilled the criteria for *combined* immunodeficiency, these were excluded. Only 6/261 patients (2.3%, 3 adults and 3 children) completely fulfilled the ESID criteria for *true* sIgMdef; 63/261 (24.1%; 44 adults and 19 children) fulfilled the criteria for *unclassified* antibody deficiency. In 192/261 patients (73.6%, 164 adults and 28 children) the diagnosis was uncertain (*possible* sIgMdef), due to incomplete laboratory data (**Figure 3.1**).

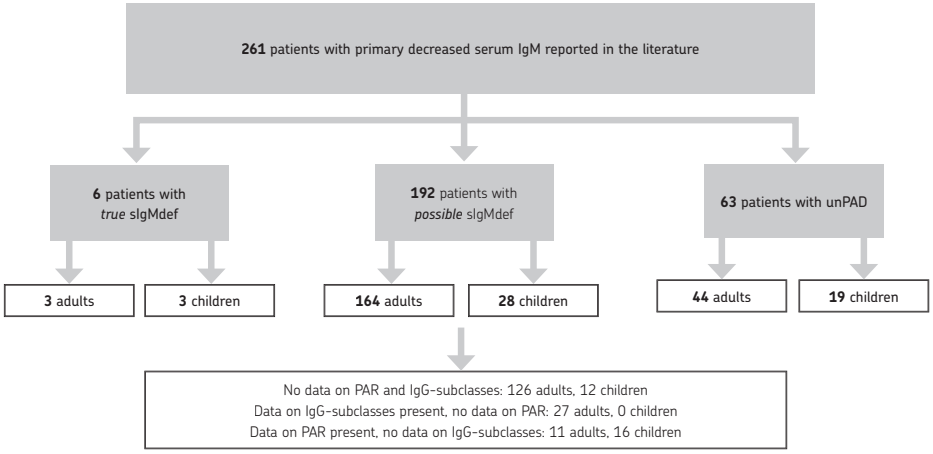


Figure 3.1. Patients with truly selective primary IgM deficiency in the literature (according to the ESID Registry clinical diagnosis criteria).

Figure 3.1. Abbreviations: PAR, pneumococcal antibody response; sIgMdef, selective IgM deficiency; unPAD, unclassified primary antibody deficiency.

Clinical and laboratory features of the published adult and paediatric cases with true or possible sIgMdef are summarized in **Tables 3.1 and 3.2**. Over two-thirds of both adults and children were male (57/85 adults, 67% vs. 23/31 children, 74%). Many patients presented with infectious problems (30/62 adults, 48% vs. 14/15 children, 93%). In 3/62 (5%) of the reported adults decreased IgM was identified "by accident" as part of laboratory evaluation for ischemic heart disease, hypertension and visual disturbance. 13/62 (21%) of the reported adults and 1/15 (7%) child were asymptomatic; this boy was detected during family screening. Serum IgM values were reported in 86 adults and 14 children (mean 0.23 g/l, range 0.004-0.45 g/l for adults vs. mean 0.18 g/l, range 0.00-0.36 g/l for

children). Undetectable serum IgM levels were reported in two children [12,13] and four adults [14]. Three adults and one baby were treated with intravenous immunoglobulin substitution (IVIG).

Our cross-sectional retrospective cohort

2,064 patients with isolated decreased serum IgM were identified in the laboratory system of the JBZ (July 1, 2005, to March 23, 2016): 2,034 adults and 17 children aged 6-18 years (13 children <6 years were excluded because the age-matched reference value was lower than the cut-off value of the test). The patient selection process is shown in detail in **Supplementary Figure 3.2**. 1,685/2,034 adults (83%) and 7/17 (41%) children had secondary isolated decreased serum IgM. 349/2,034 (17%) adults and 10/17 (59%) children had a *primary* form; of these, serum IgM levels were determined more than once in only 49/349 (14%) adults and 3/10 (30%) children. In 7/49 (14%) of the adults the serum IgM level normalized, yielding *persistent* isolated decreased serum IgM (*possible* or *true* sIgMdef cases) in 42 adults and 3 children.

More than half of the adults (54.8%) and all the children were male. Mean age at the date of the first serum sample with decreased IgM was 61 (range 33-86) years in the adults and 16 (range 16-17) years in the children. Mean follow-up time was 74.8 (range 20-133) months in the adults and 102.7 (range 82-119) months in the children.

Clinical and laboratory features are described in **Tables 3.2** (3 children) and **3.3** (42 adults). The onset and duration of symptoms could not be determined accurately in the medical files. 24% of the adults and two of the three children were analysed for suspected potential immunodeficiency. The others were detected during analysis for other problems, however, 22% of these adults and the one child had a history of symptoms that could be related to antibody deficiency (mainly infections). The majority (72%) of adults without such symptoms remained asymptomatic during follow-up; 28% developed symptoms that could be related to antibody deficiency. In none of the patients a family history of immunodeficiency was found in the medical charts. Only 7% (2 adults and 1 child) completely fulfilled the ESID criteria for true sIgMdef.

The first serum IgM level in possible or true sIgMdef cases ranged from <0.2 to 0.39 g/l (mean 0.30 ± 0.84) in the adults and from 0.28 to 0.38 g/l (mean 0.34 ± 0.05) in the children. First serum IgA levels were increased (>4.0 g/l) in 7 adults (17%). Serum IgE levels were determined in 6 adults and 1 child (mean 133 ± 182 U/ml; range 5-410 U/ml); they were elevated (>90 U/ml) in 2 adults. None of the patients were treated with IVIG or prophylactic antibiotics.

Table 3.1. Adult patients from the literature.

Year	Reference	Reported patients[a]	Age years/ gender
ESID criteria completely fulfilled (*true* sIgMdef)			
2009	[4]	3	79/M 39/F 55/M
ESID criteria not completely fulfilled: data on IgG subclasses and/or pneumococcal antibody responses lacking (*possible* sIgMdef)			
1967	[22]	5	Adult/M Adult/M Adult/M Adult/M Adult/F
1970	[24]	10	20/M 23/M 28/M 30/M 31/M 33/M 48/M 50/M 56/M 75/M
1973	[25]	2	22/M 20/M
1975	[17]	70	n.r.[c]
1976	[26]	2	72/M 60/M
1978	[27]	1	48/M
1981	[28]	1	21/M
1981	[29]	1	85/M
1982	[30]	1	65/M
1984	[31]	1	66/M
1986	[32]	7	58/M 73/F 71/F 53/F 29/F 30/M 48/M

	Clinical manifestation(s) that could be related to antibody deficiency[b]	Familial cases	Serum IgM level (g/l)	IVIG (yes/no)
	Asthma, myalgia, fatigue	No	0.18	No
	Recurrent respiratory infections, allergic rhinitis, asthma, myalgia	No	0.16	No
	Recurrent shingles, myalgia, arthralgia, fatigue	No	0.39	No
	Asymptomatic	Yes	0.40	No
	Asymptomatic	Yes	0.40	No
	Asymptomatic	Yes	0.45	No
	Asymptomatic	Yes	0.30	No
	Asymptomatic	Yes	0.30	No
	Bacterial infections, asthma	n.r.	0.36	No
	Allergic rhinitis	n.r.	0.41	No
	Bacterial infections, asthma	n.r.	0.42	No
	Bacterial infections, asthma, atopic dermatitis	n.r.	0.41	No
	Bacterial infections, asthma	n.r.	0.35	No
	Bacterial infections, atopic dermatitis	n.r.	0.24	No
	Asthma	n.r.	0.41	No
	Asthma	n.r.	0.43	No
	Asthma	n.r.	0.41	No
	Bacterial infections, asthma	n.r.	0.35	No
	CMV hepatitis	Yes	0.28	No
	Psittacosis	Yes	0.33	No
	Recurrent respiratory infections(59%), asymptomatic (19%)	n.r.	n.r.	No
	No	No	0.15	No
	Tuberculosis pneumonia	No	0.04	No
	Pneumonia, sepsis, rheumatic heart disease	n.r.	0.21	No
	Smallpox, pneumonia, died from infection	No	0.20	No
	No	n.r.	0.17	No
	No	n.r.	0.01	No
	Stomach leiomyoma	n.r.	0.08	No
	Urinary tract infection, pulmonary tuberculosis	n.r.	0.20	No
	Urinary tract infection, respiratory infection	n.r.	0.14	No
	Urinary tract infection, pneumonia	n.r.	0.11	No
	Urinary tract infection, rheumatoid arthritis	n.r.	0.17	No
	Urinary tract infection, respiratory infection, SLE	n.r.	0.25	No
	Urinary tract infection, SLE	n.r.	0.06	No
	Pneumonia	n.r.	0.10	No

Table 3.1. Continued.

Year	Reference	Reported patients[a]	Age years/ gender
1987	[33]	4	44/F 62/F 60/F 51/F
1992	[34]	6	50/M 57/M 22/M 34/M 57/M 37/F
2004	[35]	1	23/M
2006	[5]	23	Unknown[d]
2009	[4]	5	69/M 44/F 44/F 76/M 46/F
2009	[36]	2	n.r.
2015	[37]	1	52/M
2016	[2]	11	57/M 45/M 48/M 50/F 32/M 55/F 63/M 57/M 48/M 50/M 30/M
2016	[14]	10	Unkown[e]

Table 3.1. The 3 adults with true and 164 adults with possible selective primary IgM deficiency from the literature (definition of true selective IgM deficiency (sIgMdef) according to the ESID registry clinical diagnosis criteria). [a]Only reported patients fulfilling the criteria for reported true or possible primary sIgMdef are described in this table. [b]The difference between "asymptomatic" and "no" is that "no" refers to patients who were screened for problems not related to antibody deficiency in contrast to asymptomatic patients, who had no clinical problems at all.[c]70 patients were reported without specific age indications or exact IgM levels in this paper. [d]Clinical manifestations of patients were not separately described in this paper. Mean age at diagnosis of the whole group was 54 years; 11 males, 12 females. One patient was

Clinical manifestation(s) that could be related to antibody deficiency[b]	Familial cases	Serum IgM level (g/l)	IVIG (yes/no)
SLE-like	n.r.	0.26	No
Asthma	n.r.	0.23	No
Lymphoma	n.r.	0.08	No
SLE	n.r.	0.10	No
Liver abscess, cholangitis, dermatitis	No	0.18	No
Diabetes mellitus	No	0.06	No
Streptococcal infection	No	0.32	No
Chronic tonsillitis, bronchitis, psoriasis pustulosa	No	0.01	No
Diabetes mellitus, polyarthritis	No	0.004	No
Asymptomatic	No	0.34	No
Recurrent respiratory infections, allergic rhinitis, asthma	No	0.28	Yes
n.a.	No	0.32	No
Asthma, rhinorrhea	No	0.39	No
Chronic sinusitis	No	0.27	Yes
Recurrent sinus infections, allergic rhinitis, rash	No	0.28	No
Recurrent respiratory infections	No	0.30	No
Recurrent respiratory infections, rheumatoid arthritis	No	0.39	No
n.r.	n.r.	n.r.	n.r.
CEP, pericarditis, allergic rhinitis, asthma, celiac disease	No	0.32	No
Asymptomatic	No	0.19	No
Urinary tract infection (2x)	No	0.29	No
Atopic dermatitis, allergic rhinitis, food allergy	No	0.27	No
Atopic dermatitis, allergic rhinitis	No	0.25	No
Atopic dermatitis	No	0.27	No
Asymptomatic	No	0.23	No
Asymptomatic	No	0.27	No
Asymptomatic	No	0.19	No
Asymptomatic	No	0.29	No
Asymptomatic	No	0.16	No
Asymptomatic	No	0.26	No
n.r.	n.r.	Unknown	n.r.

treated with IVIG because of refractory asthma. [e]Patient data were not separately described in this paper. Of the twenty described patients, 50% had also specific antipolysaccharide antibody deficiency, and fulfilled the criteria for 'unclassified antibody deficiency'. Therefore, these 10 patients were not included in this table. Age range of the whole group: 24 years-56 years, F:M ratio 1.1:1.0, serum IgM range: 0.04 g/l to 0.32 g/l.

Abbreviations: CEP, chronic eosinophilic pneumonia; CMV, cytomegalovirus; ESID, European Society for Immunodeficiency; F, female; Ig, immunoglobulin; IVIG, intravenous immunoglobulin; M, male; n.a., not applicable; n.r., not reported; SLE, systemic lupus erythematosus.

Table 3.2. Paediatric patients from the literature and our cohort.

Year	Reference	Reported patients	Age (years/gender)
ESID criteria completely fulfilled (*true* sIgMdef)			
Our cohort			16/M
2008	[6]	2	10/M
			12/M
2009	[38]	1	6/M
Data on IgG subclasses present, but no data on pneumococcal antibody responses (*possible* sIgMdef)			
No cases			
Data on pneumococcal antibody responses present, but no data on IgG subclasses (*possible* sIgMdef)			
2013	[39]	16	Unknown[a]
No data on pneumococcal antibody responses and no data on IgG subclasses (*possible* sIgMdef)			
Our cohort			16/M
Our cohort			17/M
1967	[22]	1	5/M
1971	[13]	1	0/M
1973	[40]	1	2/F
1973	[25]	1	13/M
1973	[41]	2	4/M
			1/M
1986	[42]	1	16/F
1989	[12]	1	3/M
2001	[43]	1	10/M
2005	[23]	1	0/M
2009	[44]	1	6/M
2010	[45]	1	16/M

Table 3.2. The 3 paediatric patients from our cohort and 31 paediatric patients with true or possible selective primary IgM deficiency (sIgMdef) from the literature. [a]Patients were not separately described in this paper. Median age at diagnosis was 4.2 years; 10 males, 6 females.

	Clinical manifestations that could be related to antibody deficiency	Serum IgM level (g/l)	IVIG (yes/no)
	URTI, growth retardation, verrucae vulgares, RLS	0.36	No
	Recurrent otitis media	0.21	No
	Pneumonia	0.30	No
	Multiple recurrent impetigo	0.21	No
	n.r.	n.r.	n.r.
	Recurrent infections, asthma, verrucae vulgares	0.28	No
	Depression, long-lasting fatigue	0.38	No
	Meningococcal meningitis, died from infection	0.12	No
	Recurrent pseudomonas infections	0.00	No
	Recurrent otitis media, laryngitis, meningitis	0.08	No
	CMV hepatitis	0.26	No
	Meningitis	0.34	No
	Asymptomatic	0.36	No
	Disseminated molluscum contagiosum	0.04	No
	Recurrent infections	0.00	No
	Recurrent sinusitis, pneumonia, chronic staphylococci blepharitis	0.23	No
	Pseudomonas septicemia	0.12	Yes
	Chronic recurrent multifocal osteomyelitis	0.20	No
	Refractory giardiasis	0.21	No

Abbreviations: CMV, cytomegalovirus; F, female; Ig, immunoglobulin; IVIG, intravenous immunoglobulin; M, male; n.a., not applicable; n.r., not reported; URTI, upper respiratory tract infection; RLS, Raynaud-like symptoms.

Table 3.3. Adult patients from our cohort.

Patient	Age (years/ gender)	Reason(s) for determining serum IgM level
10 adults analysed for potential immunodeficiency		
ESID criteria completely fulfilled (*true* sIgMdef)		
1	54/F	Recurrent respiratory infections, asthma, AR
2	41/M	Recurrent respiratory infections, asthma
Data on IgG subclasses present, but no data on pneumococcal antibody responses (*possible* sIgMdef)		
3	33/M	Recurrent respiratory infections, asthma
4	33/F	Recurrent vaginal candidiasis, weight loss
5	68/F	Pneumonia
6	73/F	Recurrent pneumonia, bronchiectasis, AR
Data on pneumococcal antibody responses present, but no data on IgG subclasses (*possible* sIgMdef)		
7[a]	34/M	Arthralgia
No data on pneumococcal antibody responses and no data on IgG subclasses (*possible* sIgMdef)		
8	53/F	Recurrent UTI, sinusitis
9	71/M	Pneumonia, bronchiectasis
10	76/F	Non-healing ulcer on feet
7 adults diagnosed during analysis for other problems; history of symptoms that could be related to antibody deficiency		
No data on pneumococcal antibody responses and no data on IgG subclasses (*possible* sIgMdef)		
Serum IgM ordered by a neurologist		
11	45/M	Migraine
12	79/M	Polyneuropathy
Serum IgM ordered by an internist		
13	55/F	Liver test abnormalities
14	58/F	Liver test abnormalities
15	60/M	Collapsed vertebra
16	73/M	Renal insufficiency
17	51/M	Long-lasting fatigue, Q fever infection
25 adults diagnosed during analysis for other problems; no history of symptoms that could be related to antibody deficiency		
No data on pneumococcal antibody responses and no data on IgG subclasses (*possible* sIgMdef)		
Serum IgM ordered by a rheumatologist		
18	68/M	Arthralgia, RLS
19	65/M	Arthralgia, myalgia

	Manifestation(s) *during follow-up* that could be related to antibody deficiency	**First serum IgM level (g/l)**	**Last serum IgM level (g/l)**
	Long-lasting fatigue, keratitis	0.26	0.27
	-	0.23	0.26
	-	0.29	0.24
	-	0.24	0.24
	CREST-syndrome, ABPA	0.37	0.30
	Chronic sinusitis	0.36	0.29
	Erysipelas	<0.20	<0.20
	Inflammatory nodular hand osteoarthritis	0.26	0.24
	-	0.26	0.22
	Depression, bronchiectasis, UTI	<0.20	<0.20
	-	0.24	0.25
	Psoriasis	0.39	0.32
	-	0.38	0.31
	-	0.35	0.32
	-	0.23	0.23
	Chronic Q fever	<0.20	0.21
	-	0.26	0.33
	Cholecystitis, pharyngitis, infected hematoma	0.28	0.27
	-	0.28	0.26

Table 3.3. Continued.

Patient	Age (years/ gender)	Reason(s) for determining serum IgM level
20	75/F	Raynaud-like symptoms
21	51/M	Arthritis urica
Serum IgM ordered by an internist		
22	67/F	Hypoparathyroidism, hypothyroidism
23	70/M	Liver test abnormalities
24	62/F	Weight loss
25	52/F	Micro-albuminuria, hypothyroidism
26	43/F	Splenic infarcts, abdominal pain
27	55/M	Haematuria, recurrent kidney stones
28	71/F	Renal insufficiency
29	45/M	Renal insufficiency
30	69/M	Renal insufficiency
Serum IgM ordered by a neurologist		
31	66/M	Polyneuropathy
32	67/F	Polyneuropathy
33	68/M	Polyneuropathy
34	72/F	Polyneuropathy
35	73/F	Polyneuropathy
36	74/F	Polyneuropathy
37	74/M	Polyneuropathy
38	58/F	Polyneuropathy
39	84/M	Polyneuropathy
40	86/M	Polyneuropathy
41	46/M	Polyneuropathy
42	63/M	Polyneuropathy

Table 3.3. The 42 adult patients with true or possible selective primary IgM deficiency (sIgMdef) from our cohort. [a]This patient was diagnosed during analysis for rheumatoid arthritis. He was referred to a university centre elsewhere for analysis for potential immunodeficiency when a persistent decreased IgM level was discovered.

Manifestation(s) *during follow-up* that could be related to antibody deficiency	**First serum IgM level (g/l)**	**Last serum IgM level (g/l)**
Basal cell carcinoma	< 0.20	0.22
Inflammatory arthritis	0.38	0.30
Abscess in thigh, infection of right hip	0.27	0.27
-	0.26	< 0.2
-	0.37	0.30
Chronic Q fever	0.22	0.27
-	0.38	0.23
UTI, respiratory infection, cervical lymphadenopathy	0.35	0.37
-	0.32	0.31
-	0.37	0.32
-	0.37	0.32
-	0.36	0.31
-	0.32	0.31
Nodular basal cell carcinoma	0.39	0.37
-	0.39	0.39
-	0.32	0.36
-	0.37	0.36
-	0.33	0.37
-	0.36	0.39
-	0.37	0.36
-	0.32	0.36
-	0.32	0.23
-	0.35	0.27

Abbreviations: ABPA, allergic bronchopulmonal aspergillosis; AR, allergic rhinitis; CREST, calcinosis, raynaud's phenomenon, esophageal dysmotility, sclerodactyly, teleangiectasia; ESID, European society for immunodeficiencies; F, female; Ig, immunoglobulin; M, male; RLS, Raynaud-like symptoms; UTI, urinary tract infection.

DISCUSSION

We studied true sIgMdef (according to the ESID diagnostic criteria) by reviewing the literature and by analysing decreased serum IgM in our secondary hospital population. Our main finding is that *true* sIgMdef is probably very rare. Unfortunately, when a decreased serum IgM level is found, it is rarely fully analysed. In most cases in our cohort serum IgM levels were determined only once (86%). When proven persistently decreased, further immunological analysis is often not performed (data on IgG-subclasses and/or vaccination responses were lacking in 74% of the literature cases and 93% of the cases in our cohort). Also, different criteria for 'selective IgM deficiency' are used in the literature; in a quarter of the cases, the deficiency is not 'selective', other immunological abnormalities were present. Eight literature cases even showed clinical and/or laboratory signs fitting combined immunodeficiency; these should not be classified as a form of 'predominantly antibody deficiency'. Sixty-three (24%) literature cases fitted the ESID classification 'unclassified antibody deficiency'. These patients with concomitant defects in specific antibody production (SPAD) and/or IgG-subclass deficiencies may be at risk of more severe and frequent infections, comparable to the increased number of lower respiratory tract infections and bronchiectasis in patients with IgA deficiency in combination with IgG-subclass deficiency and/or SPAD [15]. Patients with recurrent and/or severe infections and decreased serum IgM levels in combination with SPAD, have been described to benefit from immunoglobulin treatment [4,16].

Routine determination of serum IgM is advised in many medical protocols, mainly for adults; we showed in our cohort that this leads to many incidental findings of decreased serum IgM. The relatively common finding of a low serum IgM level in – immunologically speaking – asymptomatic adults (see **Table 3.3**), often not followed by further evaluation, warrants re-evaluation of these medical protocols. In our cohort, secondary decreased serum IgM was 5 times more prevalent in adults and 2.5 times more prevalent in children than the primary form. Hobbs et al. reported that secondary decreased IgM was 20 times more prevalent than the primary form in 1975 [17]. This may be explained by the fact that age-related reference values have changed over the years, as the sensitivity of the methods used to measure serum IgM increased (Hobbs et al. <0.47 g/l >3 years, our cohort <0.21 g/l <6 years, <0.13 g/l <16 years and <0.40 g/l ≥ 16 years). Anyway, the first reaction to finding a low IgM should be to exclude a secondary cause.

The fact that only a few incidental findings of decreased serum IgM were followed by further evaluation in our cohort, suggests that the perceived medical problems were mild. Most of our incidentally diagnosed cases with true or possible sIgMdef did not have a history of symptoms related to antibody deficiency (76%), and that often remained to be the case during follow-up (72%) (on the other hand, 28% later developed symptoms that could be related to antibody deficiency). The higher prevalence of various associated diseases in the literature cases [1] is probably related to the fact that these patients had been referred to specialized allergy and immunology clinics [4–6].

Interestingly, possible or true sIgMdef was more frequently observed in males in our cohort. This parallels the observed male predominance in the literature. However, also among healthy controls low IgM levels are more common in males [18–21], and there are some reports of low serum IgM levels among fathers of patients [22,23]. It would be of interest to investigate this gender difference further.

The limitation of our study is of course its retrospective design. We collected our cohort data from the medical files, which were not collected with a research purpose in mind. Therefore, we could not correct for environmental factors and genetic polymorphisms that may influence serum IgM levels [3]. However, although very interesting on a population basis, these factors are probably not very helpful in directing decisions regarding individual patient care in the doctor's consulting room.

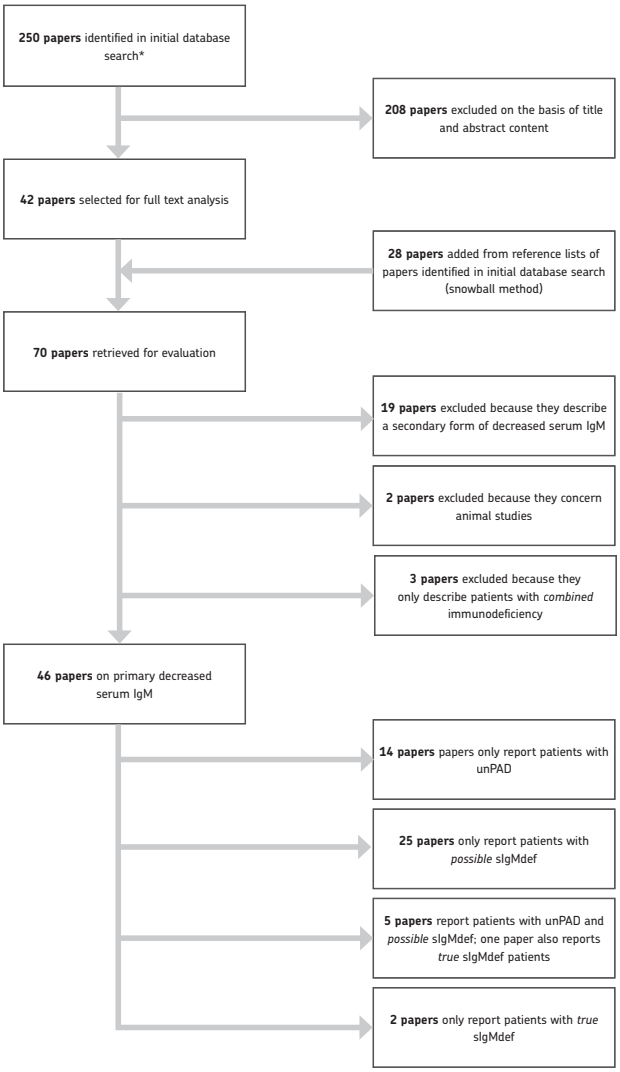
In conclusion, our review of the literature and retrospective secondary centre cohort study on decreased serum IgM, illustrate the challenge of determining the clinical significance of a serum sample with decreased IgM. The diagnosis could rarely be made with certainty, but *truly selective primary* IgM deficiency is probably very rare. Our strict definitions and thorough analysis of the available information have yielded the largest cohort study so far. Still a larger cohort of true sIgMdef patients is needed to fully explore the clinical consequences; the ESID online Registry would be a good tool for this.

REFERENCES

1. Louis AG, Gupta S. Primary selective IgM deficiency: an ignored immunodeficiency. Clin Rev Allergy Immunol. 2014 Apr;46(2):104–11.
2. Entezari N, Adab Z, Zeydi M, Saghafi S, Jamali M, Kardar GA, et al. The prevalence of Selective Immunoglobulin M Deficiency (SIgMD) in Iranian volunteer blood donors. Hum Immunol. 2016 Jan;77(1):7–11.
3. Yang M, Wu Y, Lu Y, Liu C, Sun J, Liao M, et al. Genome-wide scan identifies variant in TNFSF13 associated with serum IgM in a healthy Chinese male population. PLoS One. 2012;7(10):e47990.
4. Yel L, Ramanuja S, Gupta S. Clinical and immunological features in IgM deficiency. Int Arch Allergy Immunol. 2009;150(3):291–8.
5. Goldstein MF, Goldstein AL, Dunsky EH, Dvorin DJ, Belecanech GA, Shamir K. Selective IgM immunodeficiency: retrospective analysis of 36 adult patients with review of the literature. Ann Allergy Asthma Immunol. 2006 Dec;97(6):717–30.
6. Goldstein MF, Goldstein AL, Dunsky EH, Dvorin DJ, Belecanech GA, Shamir K. Pediatric selective IgM immunodeficiency. Clin Dev Immunol. 2008;2008:624850.
7. Guill MF, Brown DA, Ochs HD, Pyun KH, Moffitt JE. IgM deficiency: clinical spectrum and immunologic assessment. Ann Allergy. 1989 Jun;62(6):547–52.
8. Hassanein HA, Elbadry MI. Selective immunoglobulin M deficiency in an adult with miliary tuberculosis: A clinically interesting coexistence. A case report and review of the literature. Int J mycobacteriology. 2016 Mar;5(1):106–10.
9. Kanariou M, Petridou E, Liatsis M, Revinthi K, Mandalenaki-Lambrou K, Trichopoulos D. Age patterns of immunoglobulins G, A & M in healthy children and the influence of breast feeding and vaccination status. Pediatr Allergy Immunol. 1995 Feb;6(1):24–9.
10. de Vries E. Patient-centred screening for primary immunodeficiency, a multi-stage diagnostic protocol designed for non-immunologists: 2011 update. Clin Exp Immunol. 2012 Jan;167(1):108–19.
11. www.umcutrecht.nl/subsite/medische-immunologie/diagnostiek. No Title.
12. Raziuddin S, Elawad ME, Benjamin B. T-cell abnormalities in antibody deficiency syndromes. Scand J Immunol. 1989 Oct;30(4):419–24.
13. Faulk WP, Kiyasu WS, Cooper MD, Fudenberg HH. Deficiency of IgM. Pediatrics. 1971 Feb;47(2):399–404.
14. Louis AG, Agrawal S, Gupta S. Analysis of subsets of B cells, Breg, CD4Treg and CD8Treg cells in adult patients with primary selective IgM deficiency. Am J Clin Exp Immunol. 2016;5(1):21–32.
15. Aghamohammadi A, Cheraghi T, Gharagozlou M, Movahedi M, Rezaei N, Yeganeh M, et al. IgA deficiency: correlation between clinical and immunological phenotypes. J Clin Immunol. 2009 Jan;29(1):130–6.
16. Stoelinga GB, van Munster PJ. Antibody deficiency syndrome and autoimmune haemolytic anaemia in a boy with isolated IgM deficiency dysimmunoglobulinaemia type 5. Acta Paediatr Scand. 1969 Jul;58(4):352–62.
17. Hobbs JR. IgM deficiency. Birth Defects Orig Artic Ser. 1975;11(1):112–6.
18. Kacprazak-Bergman I. Sexual dimorphism of heritability of immunoglobulin levels. Ann Hum Biol. 1994;21(6):563–9.
19. Hatagima A, Cabello PH, Krieger H. Causal analysis of the variability of IgA, IgG, and IgM immunoglobulin levels. Hum Biol. 1999 Apr;71(2):219–29.

20. Kardar GA, Shams SH, Pourpak Z, Moin M. Normal value of immunoglobulins IgA, IgG, and IgM in Iranian healthy adults, measured by nephelometry. J Immunoassay Immunochem. 2003;24(4):359–67.
21. Cassidy JT, Nordby GL. Human serum immunoglobulin concentrations: prevalence of immunoglobulin deficiencies. J Allergy Clin Immunol. 1975 Jan;55(1):35–48.
22. Hobbs JR, Milner RD, Watt PJ. Gamma-M deficiency predisposing to meningococcal septicaemia. Br Med J. 1967 Dec;4(5579):583–6.
23. Zaka-ur-Rab Z, Gupta P. Pseudomonas septicemia in selective IgM deficiency. Vol. 42, Indian pediatrics. India; 2005. p. 961–2.
24. Kaufman HS, Hobbs JR. Immunoglobulin deficiencies in an atopic population. Lancet (London, England). 1970 Nov;2(7682):1061–3.
25. Silver HK, Shuster J, Gold P, Freedman SO. Leukopenia, leukoagglutinins, and low IgM in a family with severe febrile illnesses. Clin Immunol Immunopathol. 1973 Jan;1(2):220–9.
26. Ross IN, Thompson RA. Severe selective IgM deficiency. J Clin Pathol. 1976 Sep;29(9):773–7.
27. Dworzack DL, Murray CM, Hodges GR, Barnes WG. Community-acquired bacteremic Achromobacter xylosoxidans type IIIa pneumonia in a patient with idiopathic IgM deficiency. Am J Clin Pathol. 1978 Oct;70(4):712–7.
28. Brilliant LB, Nakano JH, Kitamura T, Hodakevic LN, Bharucha PB. Occupationally-acquired smallpox in an IgM-deficient health worker. Bull World Health Organ. 1981;59(1):99–106.
29. Endoh M, Kaneshige H, Tomino Y, Nomoto Y, Sakai H, Arimori S. Selective IgM deficiency: a case study. Tokai J Exp Clin Med. 1981 Jul;6(3):327–31.
30. Karsh J, Watts CS, Osterland CK. Selective immunoglobulin M deficiency in an adult: assessment of immunoglobulin production by peripheral blood lymphocytes in vitro. Clin Immunol Immunopathol. 1982 Dec;25(3):386–94.
31. Matsushita S, Inoue T, Okubo H. A case of selective IgM deficiency: isotype-specific suppressor T lymphocytes. Jpn J Med. 1984 May;23(2):149–51.
32. Inoue T, Okumura Y, Shirama M, Ishibashi H, Kashiwagi S, Okubo H. Selective partial IgM deficiency: functional assessment of T and B lymphocytes in vitro. J Clin Immunol. 1986 Mar;6(2):130–5.
33. Ohno T, Inaba M, Kuribayashi K, Masuda T, Kanoh T, Uchino H. Selective IgM deficiency in adults: phenotypically and functionally altered profiles of peripheral blood lymphocytes. Clin Exp Immunol. 1987 Jun;68(3):630–7.
34. Yamasaki T. Selective IgM deficiency: functional assessment of peripheral blood lymphocytes in vitro. Intern Med. 1992 Jul;31(7):866–70.
35. Fallon KE. Inability to train, recurrent infection, and selective IgM deficiency. Clin J Sport Med Off J Can Acad Sport Med. 2004 Nov;14(6):357–9.
36. Kutukculer N, Gulez N. The outcome of patients with unclassified hypogammaglobulinemia in early childhood. Pediatr Allergy Immunol. 2009 Nov;20(7):693–8.
37. Chen S, Shamriz O, Toker O, Fridlender ZG, Tal Y. Recurrent Eosinophilic Pneumonia in a Patient with Isolated Immunoglobulin M Deficiency and Celiac Disease. Isr Med Assoc J. 2015 Aug;17(8):526–7.
38. Belgemen T, Suskan E, Dogu F, Ikinciogullari A. Selective immunoglobulin M deficiency presenting with recurrent impetigo: a case report and review of the literature. Int Arch Allergy Immunol. 2009;149(3):283–8.
39. Cipe FE, Dogu F, Guloglu D, Aytekin C, Polat M, Biyikli Z, et al. B-cell subsets in patients with transient hypogammaglobulinemia of infancy, partial IgA deficiency, and selective IgM deficiency. J Investig Allergol Clin Immunol. 2013;23(2):94–100.

40. Ostergaard PA. A girl with recurrent infections, low IgM and an abnormal chromosome number 1. Acta Paediatr Scand. 1973 Mar;62(2):211–5.
41. Jones DM, Tobin BM, Butterworth A. Three cases of meningococcal infection in a family, associated with a deficient immune response. Arch Dis Child. 1973 Sep;48(9):742–3.
42. Mayumi M, Yamaoka K, Tsutsui T, Mizue H, Doi A, Matsuyama M, et al. Selective immunoglobulin M deficiency associated with disseminated molluscum contagiosum. Eur J Pediatr. 1986 Apr;145(1–2):99–103.
43. Kiratli HK, Akar Y. Multiple recurrent hordeola associated with selective IgM deficiency. J AAPOS Off Publ Am Assoc Pediatr Ophthalmol Strabismus. 2001 Feb;5(1):60–1.
44. Makay B, Unsal E, Anal O, Gunes D, Men S, Cakmakci H, et al. Chronic recurrent multifocal osteomyelitis in a patient with selective immunoglobulin M deficiency. Rheumatol Int. 2009 May;29(7):811–5.
45. Kampitak T. Selective immunoglobulin M deficiency in a patient with refractory giardiasis. J Investig Allergol Clin Immunol. 2010;20(4):358–60.

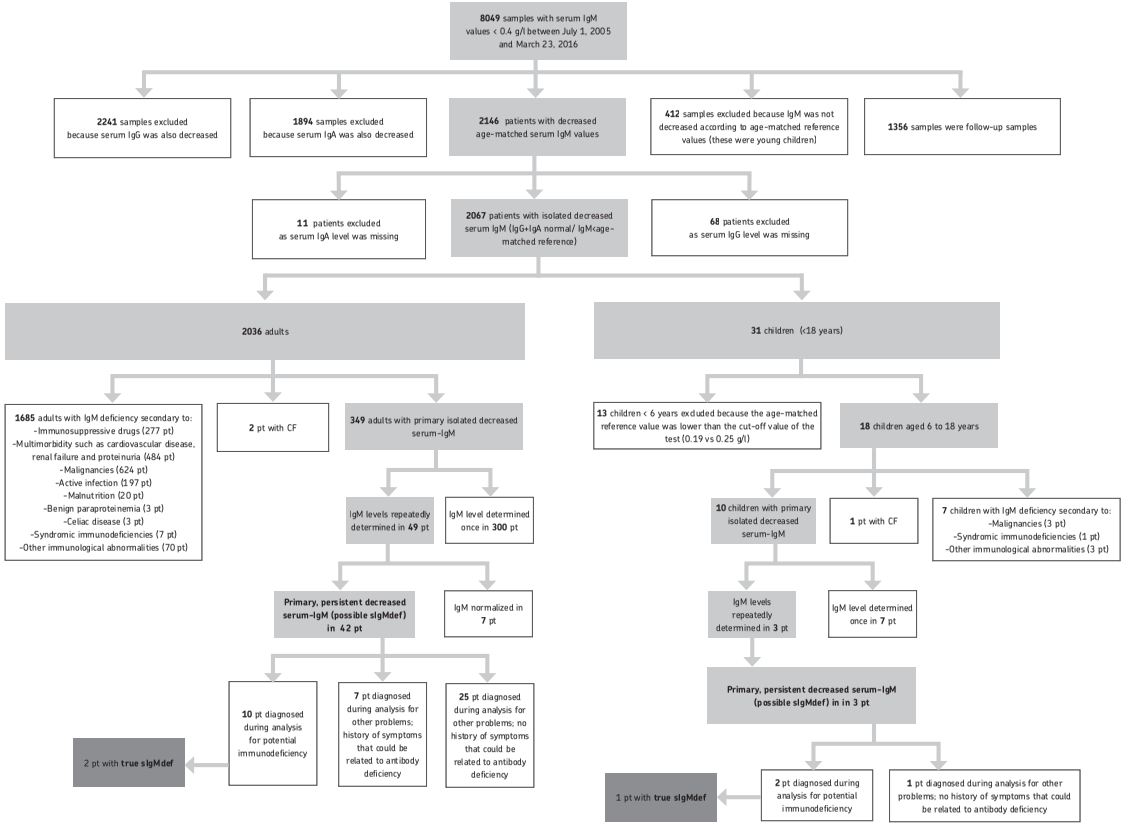


*Search strategy

Search	Query	Items found
#1	((((Deficiency) OR low)) AND (((IgM) OR Immunoglobulin M)) AND ((selective) OR isolated)	1145
#2	Immunodeficiency syndrome [MeSH Terms]	281000
#3	Combination of search #1 and #2	280
#4	Limits: English	250

Supplementary Figure 3.1. Identification of papers that report on patients with decreased serum IgM (date: 10 May 2017).

Supplementary Figure 3.1. Abbreviations: sIgMdef, selective IgM deficiency; unPAD, unclassified primary antibody deficiency.



Supplementary Figure 3.2. Patient selection.

Supplementary Figure 3.2. Abbreviations: CF, cystic fibrosis; Ig, immunoglobulin; pt, patient; sIgMdef, selective IgM deficiency.

Supplementary Table 3.1. Overview of literature.

1.Papers that report on primary decreased serum IgM (numbers of patients).				
Year	**First author**	**Reference**	**Reported patients**	***True* sIgMdef[a]**
1966	Kouvalainen	(1)	1	-
1967	Hobbs	(2)	8	-
1969	Stoelinga	(3)	1	-
1970	Kaufman	(4)	10	-
1971	Faulk	(5)	1	-
1973	Ostergaard	(6)	1	-
1973	Silver	(7)	3	-
1973	Jones	(8)	2	-
1973	Record	(9)	1	-
1975	Cassidy	(10)	1	-
1975	Hobbs	(11)	70	-
1976	Yocum	(12)	2	-
1976	Ross	(13)	2[d]	-
1978	Thong	(14)	1	-
1978	Dworzack	(15)	1	-
1981	Brilliant	(16)	1	-
1981	Endoh	(17)	1	-
1982	Karsh	(18)	1	-
1984	Matsushita	(19)	1	-
1986	Mayumi	(20)	1	-
1986	Inoue	(21)	7	-
1987	Ohno	(22)	4	-
1988	Moffitt	(23)	8	-
1989	Guill	(24)	8[e]	-
1989	Raziuddin	(25)	1[f]	-
1992	Yamasaki	(26)	6	-
2001	Kiratli	(27)	1	-
2004	Fallon	(28)	1	-
2004	Al-Herz	(29)	1	-
2005	Zaka-ur-Rab	(30)	1	-
2006	Goldstein	(31)	36	-
2007	Ideura	(32)	1	-
2008	Hong	(33)	1	-

	Possible sIgMdef[b]			unPAD[c]			
	PAR and IgGs absent	PAR absent, IgGs present	PAR present, IgGs absent	IgGsdef + impaired VR	IgGsdef	Impaired VR	IgG/ IgAdef
	-	-	-	-	-	1	-
	6	-	-	-	-	-	2
	-	-	-	-	-	1	-
	10	-	-	-	-	-	-
	1	-	-	-	-	-	-
	1	-	-	-	-	-	-
	3	-	-	-	-	-	-
	2	-	-	-	-	-	-
	-	-	-	-	-	-	1
	-	-	-	-	-	-	1
	70	-	-	-	-		-
	-	-	-	-	-	2	-
	2	-	-	-	-		-
	-	-	-	-	-	1	-
	1	-	-	-	-	-	-
	1	-	-	-	-	-	-
	1	-	-	-	-	-	-
	1	-	-	-	-	-	-
	1	-	-	-	-	-	-
	1	-	-	-	-	-	-
	7	-	-	-	-	-	-
	4	-	-	-	-	-	-
	-	-	-	3	3	-	2
	-	-	-	3	3	-	2
	1	-	-	-	-	-	-
	6	-	-	-	-	-	-
	1	-	-	-	-	-	-
	1	-	-	-	-	-	-
	-	-	-	-	-	1	-
	1	-	-	-	-	-	-
	4	19	-	-	9	-	4
	-	-	-	1	-	-	-
	-	-	-	-	1	-	-

Supplementary Table 3.1. Continued.

1.Papers that report on primary decreased serum IgM (numbers of patients).				
Year	**First author**	**Reference**	**Reported patients**	***True* sIgMdef[a]**
2008	Goldstein	(34)	2	2
2009	Belgemen	(35)	1	1
2009	Kutukculer	(36)	2	-
2009	Makay	(37)	1	-
2009	Yel	(38)	15	3
2010	Kampitak	(39)	1	-
2013	Cipe	(40)	16	-
2013	Phuphuakrat	(41)	1	-
2014	Bolia	(42)	1	-
2015	Hassanein	(43)	1	-
2015	Chen	(44)	1	-
2016	Entezari	(45)	13	-
2016	Gupta	(46)	20	-
Total			261	6

Supplementary Table 3.1. Categorized in 1) papers that report on primary decreased serum IgM; 2) papers that only report on secondary decreased serum IgM; 3) papers that concern animal studies; 4) papers that only report on combined immunodeficiency (date: 10 May 2016). [a]Definition of true sIgMdef: the ESID criteria are completely fulfilled, which means serum IgM levels are repeatedly decreased and IgG, IgA, IgG-subclasses and vaccination responses have been determined and were found to be normal for age; we consider the absence of clinical signs suggesting a T-cell defect sufficient .[b]Definition of possible sIgMdef: the diagnosis of true sIgMdef is uncertain, which means that the ESID criteria are not completely fulfilled, because data on IgG-subclasses and/or vaccination responses are lacking. [c]Definition of

	Possible sIgMdef[b]			unPAD[c]			
	PAR and IgGs absent	**PAR absent, IgGs present**	**PAR present, IgGs absent**	**IgGsdef + impaired VR**	**IgGsdef**	**Impaired VR**	**IgG/ IgAdef**
	-	-	-	-	-	-	
	-	-	-	-	-	-	
	2	-	-	-	-	-	
	1	-	-	-	-	-	
	2	2	1	2	2	3	-
	1	-	-	-	-	-	-
	-	-	16	-	-	-	-
	-	-	-	-	-	1	-
	-	-	-	-	1	-	-
	-	-	-	-	-	1	-
	1	-	-	-	-	-	-
	5	6	-	-	1	-	1
	-	-	10	-	-	10	-
		192			63		

unPAD: other abnormalities in antibodies are also present: IgG-subclass deficiency, below-normal levels of IgG or IgA, and/or impaired vaccination responses. [d]One patient fulfilled the criteria for combined immunodeficiency and was excluded. [e] The same patients as in the article from Moffitt et al. [f]Three patients fulfilled the criteria for combined immunodeficiency and were excluded.

Abbreviations: IgG/IgAdef, IgG or IgA deficiency; IgGsdef, IgG-subclass deficiency; IgGs, IgG-subclasses; PAR, pneumococcal antibody response; sIgMdef, selective IgM deficiency; unPAD; unclassified primary antibody deficiency; VR, vaccination response.

Supplementary Table 3.1. Continued.

2. Papers that only report on secondary decreased serum IgM.			
Year	**First author**	**Reference**	**Number of reported patients**
1969	Berens	(47)	1
1982	Vogelzang	(48)	1
1983	Takenaka	(49)	1
1987	Saiki	(50)	12
1992	Kondo	(51)	2
1993	Kimura	(52)	1
1995	Sivri	(53)	n.r.
2000	Iraji	(54)	1
2001	Sugita	(55)	1
2001	Takeuchi	(56)	1
2007	Gul	(57)	1
2007	Kung	(58)	2
2008	Antar	(59)	1
2011	Saini	(60)	1
2013	Arahata	(61)	1
2012	Magen	(62)	1
2013	Lim	(63)	1
2017	Lozano	(64)	6
2017	Inoue	(65)	1

Supplementary Table 3.1. Continued.

3. Papers that concern animal studies.		
Year	**First author**	**Reference**
2000	Boes	(66)
2000	Ehrenstein	(67)

Supplementary Table 3.1. Continued.

4. Papers that only report on *combined* immunodeficiency.		
Year	**First author**	**Reference**
1982	Concha	(68)
1988	Raziuddin	(69)
2017	Gharib	(70)

Secondary decreased serum IgM
Whipple's disease
Clear cell sarcoma
Prolymphocytic leukaemia
Systemic lupus erythematosus, corticosteroid treatment
Bloom's syndrome
Hashimoto's disease, serum IgM recovered upon administration of thyroid hormone
Systemic lupus erythematosus, corticosteroid treatment
Epidermodysplasia verruciformis, serum-IgM level 0.60 g/l
Chronic idiopathic thrombocytic purpura, splenectomy
Systemic lupus erythematosus, corticosteroid treatment
Epidermodysplasia verruciformis, squamous cell carcinoma
22q11.2 deletion syndrome
Malnutrition, alcohol abuses, erosive gastritis, atrophied small bowel
CD30+ cutaneous lymphoproliferative disorder
Advanced liver cirrhosis, hepatocellular carcinoma
Celiac disease
Atypical X-linked agammaglobulinemia caused by a novel BTK mutation
Clozapine
Trisomy 13

REFERENCE LIST

1. Kouvalainen K, Backman A, Rehtijarvi K. Chronic moniliasis, pyodermia and impaired capacity to form gamma-M antibodies. Ann Paediatr Fenn. 1966;12(4):256–62.
2. Hobbs JR, Milner RD, Watt PJ. Gamma-M deficiency predisposing to meningococcal septicaemia. Br Med J. 1967 Dec;4(5579):583–6.
3. Stoelinga GB, van Munster PJ. Antibody deficiency syndrome and autoimmune haemolytic anaemia in a boy with isolated IgM deficiency dysimmunoglobulinaemia type 5. Acta Paediatr Scand. 1969 Jul;58(4):352–62.
4. Kaufman HS, Hobbs JR. Immunoglobulin deficiencies in an atopic population. Lancet (London, England). 1970 Nov;2(7682):1061–3.
5. Faulk WP, Kiyasu WS, Cooper MD, Fudenberg HH. Deficiency of IgM. Pediatrics. 1971 Feb;47(2):399–404.
6. Ostergaard PA. A girl with recurrent infections, low IgM and an abnormal chromosome number 1. Acta Paediatr Scand. 1973 Mar;62(2):211–5.
7. Silver HK, Shuster J, Gold P, Freedman SO. Leukopenia, leukoagglutinins, and low IgM in a family with severe febrile illnesses. Clin Immunol Immunopathol. 1973 Jan;1(2):220–9.
8. Jones DM, Tobin BM, Butterworth A. Three cases of meningococcal infection in a family, associated with a deficient immune response. Arch Dis Child. 1973 Sep;48(9):742–3.
9. Record CO, Shilkin KB, Eddleston AL, Williams R. Intrahepatic sclerosing cholangitis associated with a familial immunodeficiency syndrome. Lancet (London, England). 1973 Jul;2(7819):18–20.
10. Cassidy JT, Nordby GL. Human serum immunoglobulin concentrations: prevalence of immunoglobulin deficiencies. J Allergy Clin Immunol. 1975 Jan;55(1):35–48.
11. Hobbs JR. IgM deficiency. Birth Defects Orig Artic Ser. 1975;11(1):112–6.
12. Yocum MW, Strong DM, Chusid MJ, Lakin JD. Selective immunoglobulin M (IgM) deficiency in two immunodeficient adults with recurrent staphylococcal pyoderma. Am J Med. 1976 Apr;60(4):486–94.
13. Ross IN, Thompson RA. Severe selective IgM deficiency. J Clin Pathol. 1976 Sep;29(9):773–7.
14. Thong YH, Maxwell GM. Primary selective deficiency of immunoglobulin M. Aust N Z J Med. 1978 Aug;8(4):436–8.
15. Dworzack DL, Murray CM, Hodges GR, Barnes WG. Community-acquired bacteremic Achromobacter xylosoxidans type IIIa pneumonia in a patient with idiopathic IgM deficiency. Am J Clin Pathol. 1978 Oct;70(4):712–7.
16. Brilliant LB, Nakano JH, Kitamura T, Hodakevic LN, Bharucha PB. Occupationally-acquired smallpox in an IgM-deficient health worker. Bull World Health Organ. 1981;59(1):99–106.
17. Endoh M, Kaneshige H, Tomino Y, Nomoto Y, Sakai H, Arimori S. Selective IgM deficiency: a case study. Tokai J Exp Clin Med. 1981 Jul;6(3):327–31.
18. Karsh J, Watts CS, Osterland CK. Selective immunoglobulin M deficiency in an adult: assessment of immunoglobulin production by peripheral blood lymphocytes in vitro. Clin Immunol Immunopathol. 1982 Dec;25(3):386–94.
19. Matsushita S, Inoue T, Okubo H. A case of selective IgM deficiency: isotype-specific suppressor T lymphocytes. Jpn J Med. 1984 May;23(2):149–51.
20. Mayumi M, Yamaoka K, Tsutsui T, Mizue H, Doi A, Matsuyama M, et al. Selective immunoglobulin M deficiency associated with disseminated molluscum contagiosum. Eur J Pediatr. 1986 Apr;145(1–2):99–103.

21. Inoue T, Okumura Y, Shirama M, Ishibashi H, Kashiwagi S, Okubo H. Selective partial IgM deficiency: functional assessment of T and B lymphocytes in vitro. J Clin Immunol. 1986 Mar;6(2):130–5.
22. Ohno T, Inaba M, Kuribayashi K, Masuda T, Kanoh T, Uchino H. Selective IgM deficiency in adults: phenotypically and functionally altered profiles of peripheral blood lymphocytes. Clin Exp Immunol. 1987 Jun;68(3):630–7.
23. Moffitt JE, Guill MF, Wray BB, Brown DA, Peacocke NW, Ades EW. Effect of interleukin-2 and mitogen on in vitro immunoglobulin production by peripheral blood lymphocytes from patients with selective IgM deficiency. Ann Allergy. 1988 Dec;61(6):424–7.
24. Guill MF, Brown DA, Ochs HD, Pyun KH, Moffitt JE. IgM deficiency: clinical spectrum and immunologic assessment. Ann Allergy. 1989 Jun;62(6):547–52.
25. Raziuddin S, Elawad ME, Benjamin B. T-cell abnormalities in antibody deficiency syndromes. Scand J Immunol. 1989 Oct;30(4):419–24.
26. Yamasaki T. Selective IgM deficiency: functional assessment of peripheral blood lymphocytes in vitro. Intern Med. 1992 Jul;31(7):866–70.
27. Kiratli HK, Akar Y. Multiple recurrent hordeola associated with selective IgM deficiency. J AAPOS Off Publ Am Assoc Pediatr Ophthalmol Strabismus. 2001 Feb;5(1):60–1.
28. Fallon KE. Inability to train, recurrent infection, and selective IgM deficiency. Clin J Sport Med Off J Can Acad Sport Med. 2004 Nov;14(6):357–9.
29. Al-Herz W, McGeady SJ, Gripp KW. 22q11.2 deletion syndrome and selective IgM deficiency: an association of a common chromosomal abnormality with a rare immunodeficiency. Vol. 127A, American journal of medical genetics. Part A. United States; 2004. p. 99–100.
30. Zaka-ur-Rab Z, Gupta P. Pseudomonas septicemia in selective IgM deficiency. Vol. 42, Indian pediatrics. India; 2005. p. 961–2.
31. Goldstein MF, Goldstein AL, Dunsky EH, Dvorin DJ, Belecanech GA, Shamir K. Selective IgM immunodeficiency: retrospective analysis of 36 adult patients with review of the literature. Ann Allergy Asthma Immunol. 2006 Dec;97(6):717–30.
32. Ideura G, Agematsu K, Komatsu Y, Hatayama O, Yasuo M, Tsushima K, et al. Selective IgM deficiency accompanied with IgG4 deficiency, dermal complications and a bronchial polyp. Allergol Int. 2008 Mar;57(1):99–105.
33. Hong R, Gupta S. Selective immunoglobulin M deficiency in an adult with Streptococcus pneumoniae sepsis and invasive aspergillosis. J Investig Allergol Clin Immunol. 2008;18(3):214–8.
34. Goldstein MF, Goldstein AL, Dunsky EH, Dvorin DJ, Belecanech GA, Shamir K. Pediatric selective IgM immunodeficiency. Clin Dev Immunol. 2008;2008:624850.
35. Belgemen T, Suskan E, Dogu F, Ikinciogullari A. Selective immunoglobulin M deficiency presenting with recurrent impetigo: a case report and review of the literature. Int Arch Allergy Immunol. 2009;149(3):283–8.
36. Kutukculer N, Gulez N. The outcome of patients with unclassified hypogammaglobulinemia in early childhood. Pediatr Allergy Immunol. 2009 Nov;20(7):693–8.
37. Makay B, Unsal E, Anal O, Gunes D, Men S, Cakmakci H, et al. Chronic recurrent multifocal osteomyelitis in a patient with selective immunoglobulin M deficiency. Rheumatol Int. 2009 May;29(7):811–5.
38. Yel L, Ramanuja S, Gupta S. Clinical and immunological features in IgM deficiency. Int Arch Allergy Immunol. 2009;150(3):291–8.
39. Kampitak T. Selective immunoglobulin M deficiency in a patient with refractory giardiasis. J Investig Allergol Clin Immunol. 2010;20(4):358–60.

40. Cipe FE, Dogu F, Guloglu D, Aytekin C, Polat M, Biyikli Z, et al. B-cell subsets in patients with transient hypogammaglobulinemia of infancy, partial IgA deficiency, and selective IgM deficiency. J Investig Allergol Clin Immunol. 2013;23(2):94–100.
41. Phuphuakrat A, Ngamjanyaporn P, Nantiruj K, Luangwedchakarn V, Malathum K. Selective IgM deficiency in an adult presenting with Streptococcus pneumoniae septic arthritis. J Microbiol Immunol Infect. 2016 Feb;49(1):150–3.
42. Bolia R, Misra DP, Aggarwal A, Srivastava A. Paediatric selective IgM deficiency and IgG4 deficiency: an extremely unusual association. BMJ Case Rep. 2014 Sep;2014.
43. Hassanein HA, Elbadry MI. Selective immunoglobulin M deficiency in an adult with miliary tuberculosis: A clinically interesting coexistence. A case report and review of the literature. Int J mycobacteriology. 2016 Mar;5(1):106–10.
44. Chen S, Shamriz O, Toker O, Fridlender ZG, Tal Y. Recurrent Eosinophilic Pneumonia in a Patient with Isolated Immunoglobulin M Deficiency and Celiac Disease. Isr Med Assoc J. 2015 Aug;17(8):526–7.
45. Entezari N, Adab Z, Zeydi M, Saghafi S, Jamali M, Kardar GA, et al. The prevalence of Selective Immunoglobulin M Deficiency (SIgMD) in Iranian volunteer blood donors. Hum Immunol. 2016 Jan;77(1):7–11.
46. Louis AG, Agrawal S, Gupta S. Analysis of subsets of B cells, Breg, CD4Treg and CD8Treg cells in adult patients with primary selective IgM deficiency. Am J Clin Exp Immunol. 2016;5(1):21–32.
47. Berens SC, Cohen RA, Schwabe AD. Diagnostic problems of partially treated Whipple's disease. Report of a case with isolated deficiency of immunoglobulin M. Calif Med. 1969 Jun;110(6):477–81.
48. Vogelzang NJ, Corwin H, Finlay JL, Pellettiere EV 2nd, Luskin AT, Di Camelli RF, et al. Clear cell sarcoma and selective IgM deficiency: a case report. Cancer. 1982 Jan;49(2):234–8.
49. Takenaka T, Nakamine H, Nishihara T, Tsuda T, Tsujimoto M, Maeda J. Prolymphocytic leukemia with IgM hypogammaglobulinemia. Am J Clin Pathol. 1983 Aug;80(2):237–42.
50. Saiki O, Saeki Y, Tanaka T, Doi S, Hara H, Negoro S, et al. Development of selective IgM deficiency in systemic lupus erythematosus patients with disease of long duration. Arthritis Rheum. 1987 Nov;30(11):1289–92.
51. Kondo N, Ozawa T, Kato Y, Motoyoshi F, Kasahara K, Kameyama T, et al. Reduced secreted mu mRNA synthesis in selective IgM deficiency of Bloom's syndrome. Clin Exp Immunol. 1992 Apr;88(1):35–40.
52. Kimura S, Tanigawa M, Nakahashi Y, Inoue M, Yamamura Y, Kato H, et al. Selective IgM deficiency in a patient with Hashimoto's disease. Intern Med. 1993 Apr;32(4):302–7.
53. Sivri A, Hascelik Z. IgM deficiency in systemic lupus erythematosus patients. Vol. 38, Arthritis and rheumatism. United States; 1995. p. 1713.
54. Iraji F, Faghihi G. Epidermodysplasia verruciformis: association with isolated IgM deficiency and response to treatment with acitretin. Clin Exp Dermatol. 2000 Jan;25(1):41–3.
55. Sugita K, Eguchi M. Chronic idiopathic thrombocytic purpura in a young male patient with isolated IgM deficiency. Vol. 73, International journal of hematology. Japan; 2001. p. 532–3.
56. Takeuchi T, Nakagawa T, Maeda Y, Hirano S, Sasaki-Hayashi M, Makino S, et al. Functional defect of B lymphocytes in a patient with selective IgM deficiency associated with systemic lupus erythematosus. Autoimmunity. 2001;34(2):115–22.
57. Gul U, Soylu S, Yavuzer R. Epidermodysplasia verruciformis associated with isolated IgM deficiency. Indian J Dermatol Venereol Leprol. 2007;73(6):420–2.
58. Kung S-J, Gripp KW, Stephan MJ, Fairchok MP, McGeady SJ. Selective IgM deficiency and 22q11.2 deletion syndrome. Ann Allergy Asthma Immunol. 2007 Jul;99(1):87–92.
59. Antar M, Lamarche J, Peguero A, Reiss A, Cole S. A case of selective immunoglobulin M deficiency and autoimmune glomerulonephritis. Clin Exp Nephrol. 2008 Aug;12(4):300–4.

60. Saini S, Dettore AJ, Bhambhani KJ, Buck S, Poulik J, Savasan S. Selective IgM deficiency in CD30+ cutaneous lymphoproliferative disorder. J Pediatr Hematol Oncol. 2011 May;33(4):e156-9.
61. Arahata M, Tajiri K, Nomoto K, Tsuneyama K, Minami S, Shimizu Y. A novel type of selective immunoglobulin m deficiency in a patient with autoimmune liver cirrhosis with recurrent hepatocellular carcinoma: a case report and review of the literature. Int Arch Allergy Immunol. 2013;161(1):91–6.
62. Magen E, Feldman V, Joseph M, Israel H. Symptomatic Secondary Selective IgM Immunodeficiency in Adult Man with Undiagnosed Celiac Disease. Case reports Immunol. 2012;2012:684247.
63. Lim L-M, Chang J-M, Wang I-F, Chang W-C, Hwang D-Y, Chen H-C. Atypical X-linked agammaglobulinaemia caused by a novel BTK mutation in a selective immunoglobulin M deficiency patient. BMC Pediatr. 2013 Sep;13:150.
64. Lozano R, Marin R, Santacruz M-J, Pascual A. Selective Immunoglobulin M Deficiency Among Clozapine-Treated Patients: A Nested Case-Control Study. Prim care companion CNS Disord. 2015;17(4).
65. Inoue CN, Tanaka Y, Tabata N. Acne conglobata in a long-term survivor with trisomy 13, accompanied by selective IgM deficiency. Am J Med Genet A. 2017 May;
66. Boes M, Schmidt T, Linkemann K, Beaudette BC, Marshak-Rothstein A, Chen J. Accelerated development of IgG autoantibodies and autoimmune disease in the absence of secreted IgM. Proc Natl Acad Sci U S A. 2000 Feb;97(3):1184–9.
67. Ehrenstein MR, Cook HT, Neuberger MS. Deficiency in serum immunoglobulin (Ig)M predisposes to development of IgG autoantibodies. J Exp Med. 2000 Apr;191(7):1253–8.
68. De la Concha EG, Garcia-Rodriguez MC, Zabay JM, Laso MT, Alonso F, Bootello A, et al. Functional assessment of T and B lymphocytes in patients with selective IgM deficiency. Clin Exp Immunol. 1982 Sep;49(3):670–6.
69. Raziuddin S, Bilal N, Benjamin B. Transient T-cell abnormality in a selective IgM-immunodeficient patient with Brucella infection. Clin Immunol Immunopathol. 1988 Mar;46(3):360–7.
70. Gharib A, Louis AG, Agrawal S, Gupta S. Syndrome of selective IgM deficiency with severe T cell deficiency associated with disseminated cutaneous mycobacterium avium intracellulaire infection. Am J Clin Exp Immunol. 2015;4(2):15–27.

Chapter 4

Challenges in investigating patients with isolated decreased serum IgM - The SIMcal study

Lisanne M.A. Janssen, Roeland W.N.M. van Hout, Esther de Vries and the SIMcal consortium

Scand J Immunol. 2019; 89(6):e12763

ABSTRACT

The clinical consequences of isolated decreased serum immunoglobulin (Ig)M are not sufficiently known. Therefore, it is difficult to determine the clinical policy following such a finding. Only few reported IgM-deficient patients fulfil the European Society for Immunodeficiencies (ESID) diagnostic criteria for selective IgM deficiency (true sIgMdef), or their diagnosis is uncertain due to insufficient laboratory data (possible sIgMdef). Decreased serum IgM is often incidentally found in asymptomatic adults. The objective of our study was to further characterize true sIgMdef and to compare the European data collected through the ESID Registry community (tertiary centres) to our previously published Dutch cohort (secondary centre). Fifteen centres (12 countries) participated with 98 patients. Patients were excluded if serum IgM was only determined once (n=14), had normalized (n=8), or if they also had other immunological abnormalities (n=15). Ten patients (5 adults) completely fulfilled the ESID criteria for true sIgMdef. Age-matched cut-off values varied widely between centres; when using the ESID diagnostic protocol reference values, only 6 patients (5 adults) had true sIgMdef. Because of these small numbers, further analyses were performed in patients with true or possible sIgMdef (13 adults, 48 children). Respiratory infections were commonly reported at presentation (adults 54%, children 60%). Symptomatic adults had lower serum IgM levels (mean 0.27g/l, 95%CI 0.22-0.31) than those without symptoms (mean 0.33g/l, 95%CI 0.30-0.36; p=0.02). To be able to explore the clinical consequences of *true* sIgMdef, we should fully analyse and accurately describe those patients in whom a decreased serum IgM is found.

INTRODUCTION

The clinical consequences of isolated decreased serum immunoglobulin (Ig)M levels are not sufficiently known. Clinicians struggle with what they should do with such a finding. IgM deficiency has mainly been studied in tertiary centre cohorts, where a variety of clinical manifestations have been linked with decreased serum IgM levels, including severe or recurrent infections, atopy, autoimmunity and malignancy [1]. Only small cohorts of IgM deficient patients have been described so far [2,3,12,13,4–11]. In 2006, the largest study to date was published, reporting data from 36 patients [14]. The reported patients are almost always symptomatic and most of them presented with infections [1]. We recently showed in a secondary centre population that decreased serum IgM levels can often incidentally be found in asymptomatic adults [15]. The determination of the clinical significance of sIgMdef is not only challenged by the rarity and highly variable phenotype of this primary immunodeficiency, but also by the different criteria for 'selective IgM deficiency' that are used in the literature [4,13,14,16]. ESID has defined primary selective immunoglobulin(Ig)M deficiency (sIgMdef) as a decreased serum IgM level (repeatedly ≥ 2 SD below the mean for age) with normal levels of serum IgA, IgG and IgG subclasses, normal vaccination responses, absence of T cell defects, and absence of causative external factors (http://www.esid.org). When these criteria are completely fulfilled, we refer to this condition as 'truly selective primary IgM deficiency' (*true* sIgMdef), albeit we consider the absence of *clinical* signs suggesting a T cell defect a sufficient criterion. Only six of 261 (2%) patients described in the literature with 'IgM deficiency' completely fulfil the defined criteria for true sIgMdef [15]. For many reported patients the diagnosis is either uncertain, which means that the ESID criteria are not fulfilled completely because data on IgG subclasses and/or vaccination responses are lacking (we refer to the latter as '*possible* sIgMdef') [15], or their IgM deficiency is not selective, because other antibody abnormalities are present; these cases fit the ESID classification 'unclassified primary antibody deficiency' (*unPAD*) [3,4,17].

A larger cohort of *true* sIgMdef patients is needed to further explore the clinical consequences. Therefore, we initiated this multi-centre observational cohort study using the ESID online Database. We also compared these European data (tertiary centres) to our previously published Dutch cohort (secondary centre) [15].

MATERIALS AND METHOD

Patient Identification and Recruitment

Email messages with the proposal to participate in the SIMcal study were sent out to all members of ESID to identify as many patients known to ESID members as possible with sIgMdef. Fifteen centres agreed to participate. Of these, 11 centres had registered their patients in the ESID online Database[18]. The four centres not connected to the ESID online Database also joined the SIMcal study. All patients documented by the participating centres to have sIgMdef were eligible for analysis. Only the patients with possible and true primary sIgMdef were analysed in detail (for definitions, see introduction). In all cases, patients had given informed consent for analysis of their data. The Medical Ethical Committee Brabant approved the SIMcal study.

Data Collection

The development, ongoing management and technical database structure of the ESID online Database were described previously [18]. All participating centres entered their data in the study questionnaire, providing available demographic and clinical data (gender, date of birth, country of residence, age at diagnosis, date of diagnosis, presenting history, conditions during follow-up, pathogens, familial cases, consanguinity), as well as laboratory test results (serum IgM, IgG, IgA, and IgE levels, IgG-subclasses, T cell subsets and function, antibody responses to vaccinations, Isohemagglutinin levels, anti-nuclear antibodies (ANA) and specific IgE directed against inhalant allergens), treatment (antibiotics, immunoglobulin substitution), and follow-up period (date of the first serum sample with decreased IgM until the date of data extraction). The answers to the questionnaires were encrypted and saved on a protected server using Research Manager software developed by Cloud9 Health Solutions (Deventer, the Netherlands). For interpretation of serum immunoglobulin levels, centre-specific age-matched reference values were used. Almost all centres used immunonephelometric or immunoturbidimetric techniques (14 out of 15); in one centre radial immunodiffusion was used (Egypt). The method of data collection for the 42 adults with true or possible sIgMdef from the secondary centre has been described before [15].

Statistical Analysis

Frequency data were analysed with chi square analysis, and the Fisher exact test when expected cell values were lower than 5. Measurement data were expressed as means with standard deviations (SD) and confidence intervals (CI). Differences in measurements were tested with T-test (Welch's T-test when the variances are unequal) and ANOVA. The statistical software package used was IBM SPSS statistics version 24.

RESULTS

Data from 98 patients were reported from 15 centres in 12 different countries. Thirty-seven patients (37%) were excluded: 14 because serum IgM level was only determined once, 8 because serum IgM level had normalized, and 15 because other immunological abnormalities were also present (these patients fulfilled the criteria for unPAD).

Of the remaining 61 patients, only 10 fulfilled the ESID criteria for true sIgMdef (5 adults, 5 children), and 51 had possible sIgMdef (8 adults, 43 children) when using the age-matched cut-off values for serum IgM used by the reporting centre. In those with possible sIgMdef, the following immunological laboratory investigations were not determined: pneumococcal vaccination responses (0 adults and 20 children), IgG subclasses (1 adult, 0 children), or both (7 adults and 23 children). Cut-off values varied widely between centres (**Figure 4.1**). When ESID diagnostic protocol cut-off values for serum IgM were used [19], only 6 patients (5 adults, 1 child) had true sIgMdef, and 8 had possible sIgMdef (6 adults and 2 children).

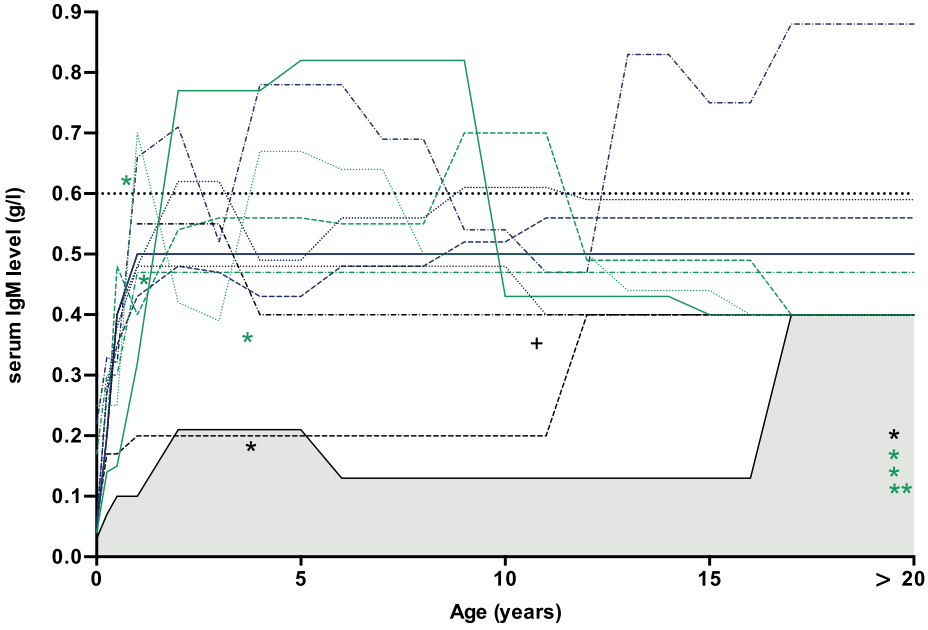


Figure 4.1. Centre-specific age-matched cut-off values of serum IgM (g/l).

Figure 4.1. Each line represents the lower limit of normal for serum IgM used by a centre. The grey area represents serum IgM levels which are decreased according to the ESID diagnostic protocol values [19]. The first serum IgM levels of the ten patients with true sIgMdef according to centre-specific cut-off values are plotted (C1,2,4 from Belgium; C3 from Iran; C5, A3 from the Netherlands; A1,2,4,5 from the Czech Republic). Of these, four patients were excluded when ESID diagnostic protocol values were used (shown in grey).

Abbreviations: ESID, European Society for Immunodeficiencies; sIgMdef, selective IgM deficiency.

Children

Analyses were done for the total group of children with possible or true primary sIgMdef (n=48). Most children were reported from Turkey (n=24), followed by Italy (n=11), Tunisia (n=4), Belgium (n=3), Iran (n=3), The Netherlands (n=1), and Spain (n=2). The mean age at the date of the first serum sample with decreased serum IgM in this possible/true sIgMdef cohort was 7 years (range 0-17 years). Mean follow-up time was 54 months (range 0-162 months). Boys predominated (79%), but there was a significant association between country and gender (Fisher exact test, 2-sided, p=0.002). The numbers of children in the various countries were too small to draw reliable conclusions from the gender data (**Figure 4.2**). Consanguinity was present in six patients (13%, n=2 male), absent in 39 (81%, n=35 male), and not reported in three (6%, n=1 male). These patients from consanguineous families were reported by Iran (2 out of 3), Italy (2 out of 11), and Turkey (2 out of 24). Familial cases were present in three patients (6%; 2 from Iran, 1 from Italy), absent in 42 (81%), and not reported in three (6%).

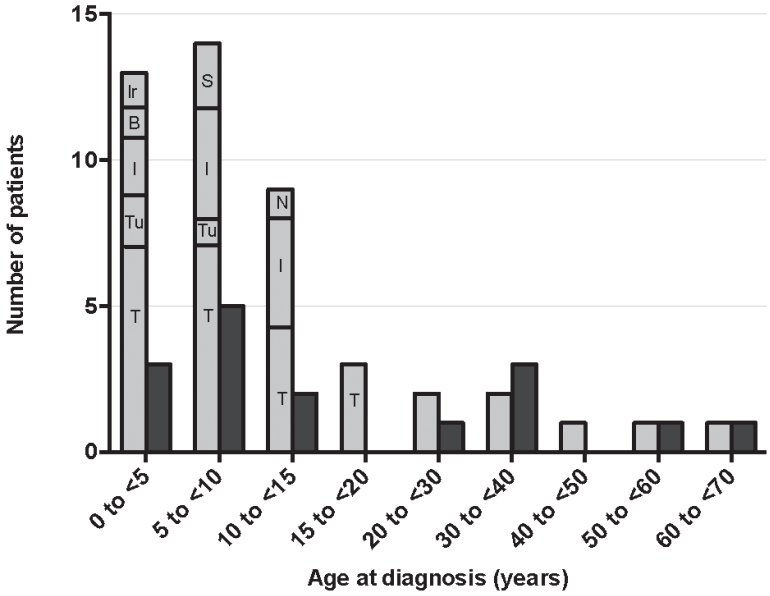


Figure 4.2. Gender distribution per age group in the patients with possible and true sIgMdef.

Figure 4.2. Light grey, male; dark grey, female. The number of children reported per country is shown for the male children. T=Turkey, Tu=Tunisia, I=Italy, B=Belgium, Ir=Iran, S=Spain, N=The Netherlands.

Recurrent respiratory infections were the most commonly reported manifestation (n=29; 60%). Other infectious manifestations included mycobacterial adenitis, skin infections, and bilateral pneumonia with an abscess. Atopic manifestations occurred in 11 children (21%), including eczema, food allergy, and asthma. An autoimmune manifestation occurred in 1 child

(2%), more specific information was not available in the database. The first serum IgM level ranged from 0.12 to 0.62 g/l (mean 0.35 g/l). In the majority of the children, IgM levels were not decreased according to the ESID diagnostic protocol values; none had undetectable levels of serum IgM (**Figure 4.3A**). Analysis of variance showed a significant effect for differences in serum IgM levels between countries ($F = 5.858$, $p=0.001$, partial $h^2 = 0.417$, **Figure 4.3B**). Especially in Belgium serum IgM values were higher and in Tunisia and Iran lower, but due to the low number of patients reported by these countries, it is difficult to interpret these results.

Mean serum IgM levels were higher in males than in females (mean 0.37 versus 0.26 g/l; $t(12.208) = 2.697$, $p=0.02$), but when the variation between countries was taken into account, this difference was no longer significant (two-way ANOVA; $F(1,37) = 2.038$, $p=0.1$). Serum IgE levels were determined in 25 children (mean 184 U/ml, range 3-1225); they were elevated (>90 U/ml) in 11 children (44%). Specific IgE(s) to ≥1 inhalant allergen were positive in 8/16 children (50%). Isohemagglutinin titres (anti-A and anti-B antibodies in the IgM class) were determined in 23 children, and low in two. Lymphocyte subsets were performed in 30 children (**Table 4.1A**). Three children (6%) were treated with intravenous immunoglobulins (IVIG), and 10 (21%) with prophylactic antibiotics.

Clinical manifestations of the children with true sIgMdef are described separately in **Table 4.1B** (see **Supplementary Table 4.1** for more details on all the children, and **Supplementary Table 4.2** for a comparison between the Turkish children (largest group) and the children from the other countries).

Adults

Thirteen adults (7 males) with true or possible sIgMdef were reported from Turkey (n=4), Czech Republic (n=4), The Netherlands (n=3), and the United Kingdom (n=2). The mean age at the date of the first serum sample with decreased IgM was 40 years (range 21-63 years). Mean follow-up time was 64 months (range 4-144 months). None of the adults had a family history of immunodeficiency (unknown in one) or consanguinity.

Clinical manifestations of the adults with true sIgMdef are described in **Table 4.2A** (for details on all the adults, see **Supplementary Table 4.1**). Increased susceptibility to infections, especially involving the respiratory tract, occurred most often (n=7). Other reported infectious manifestations included hepatitis B, meningococcal sepsis, and recurrent herpes simplex virus (HSV) encephalitis. Atopic manifestations occurred in two adults, including atopic dermatitis and allergic rhinitis. Autoimmune manifestations occurred in three (Sjogren's disease, alopecia, celiac disease). The first serum IgM level ranged from 0.10 to 0.62 g/l (mean 0.27 g/l). Serum IgE levels were determined in five adults (mean 109 U/ml, range 4-410); they were elevated (> 90 U/ml) in two. Isohemagglutinin titres were determined in four adults, and low in one. Lymphocyte subsets were performed in 9 patients (**Table 4.2B**), all fell within the normal range. None of the adults were treated with IVIG, three (23%) with prophylactic antibiotics.

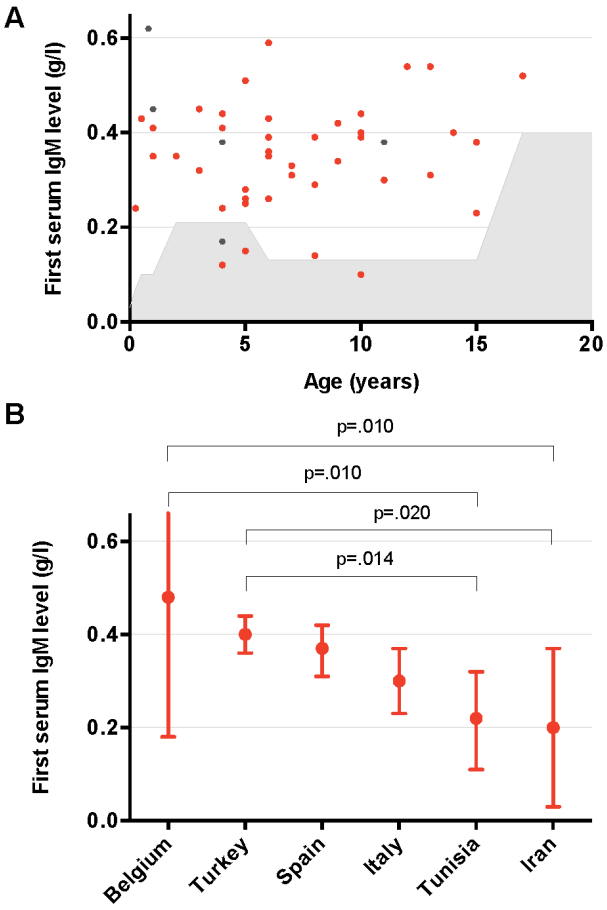


Figure 4.3. First serum IgM levels in the children from the tertiary centre cohort.

Figure 4.3. First serum IgM levels (y-axis) and age at the date of the first serum sample (x-axis). The grey dots represent the 5 children with true sIgMdef, and the red dots the 43 children with possible sIgMdef. The grey area in the graph represents decreased IgM levels according to the ESID diagnostic protocol values [18]. B. Mean first serum IgM levels + 95%CI in the different countries. Abbreviations: sIgMdef, selective IgM deficiency.

Table 4.1. Children.

A. Lymphocyte subsets in children with true (n=5) or possible sIgMdef (n=25).											
Patient	**Age[a] (years)**	**CD3+ T cells** x 10^e9/l	%	**CD4+ T cells** x 10^e9/l	%	**CD8+ T cells** x 10^e9/l	%	**CD19+ B cells** x 10^e9/l	%	**CD3-CD16+ CD56+ NK cells** x 10^e9/l	%
True sIgMdef											
C1	0[b]	2.5		1.2		1.2		0.7		0.2	
C2	1	3.8		2.4		1.3		2		0.4	
C3	4		45		33		11		33		17
C4	4	1.8		0.9		0.8		0.7		0.24	
C5	11	1.6		0.8		0.6		NA		NA	
Possible sIgMdef											
C6	0[c]		70		***25***		42		24		7
C7	0[d]		64		36		24		28		8
C9	1		67		39		25		21		7
C10	2		58		28		22		21		15
C13	4	1.9		1.0		0.8		0.2		0.2	
C17	5		75		53		21		15		9
C18	5		72		47		23		22		5
C20	5		63		38		21		16		16
C22	5		90		52		38		***3***		11
C23	5		81		49		26		13		6
C26	6		75		30		34		13		10
C28	6		75		31		38		14		7
C29	7	1.9		1.0		0.7		0.5		0.36	
C31	8		78		58		17		9		12
C32	8		73		36		34		15		10
C33	8		79		39		34		11		9
C34	9		57		35		12		13		24
C36	10		80		51		25		12		8
C37	10		58		26		30		16		18
C38	10		73		43		27		15		12
C39	10		68		43		23		16		14
C40	11		73		31		29		17		10
C41	11		73		38		17		7		16
C47	15		76		30		43		9		15
C48	17		77		39		29		7		15

Table 4.1. Continued.

B. Clinical manifestations of the children with true sIgMdef (n=5).		
Patient	**Age[a] (years)/gender**	**Clinical manifestations**
C1	0/M	Recurrent pneumonia
C2	1/M	Recurrent ENT infections
C3	4/F	Complicated atypical mycobacterial adenitis, recurrent respiratory infections
C4	4/F	Atopic dermatitis, eczema, food allergy, asthma, warts
C5	11/F	Severe eczema

Table 4.1. [a]Age at first sample collection. [b]8 months. [c]6 months. [d]7 months. [e]This serum IgM level is decreased according to the age-matched reference values used by this centre.
Abbreviations: AB, prophylactic antibiotics; C, child; ENT, ear-nose-throat; F, female; IgM, immunoglobulin M; IVIG, intravenous immunoglobulins; M, male; n.r, not reported; sIgMdef, selective IgM deficiency.
Reference ranges from: Schatorjé et al. Scand J Immunol 2011;74(5):502-10[35].

Table 4.2. Adults.

A. Clinical manifestations of the adults with true sIgMdef (n=5).		
Patient	**Age[a] (years)/gender**	**Clinical manifestations**
A1	36/F	Atopic dermatitis, allergic rhinitis, sinusitis
A2	38/F	Bronchitis, nasopharyngitis, chronic hepatitis B
A3	50/F	Bronchiectasis, celiac disease, fatigue, recurrent respiratory infections
A4	55/M	Vertebral pain syndrome
A5	63/F	Sjogren's syndrome, alopecia, multiple lung cysts, fatigue

Familial cases	First- and last serum IgM (g/l)	Treatment	Follow-up period (months)
No	0.62[e], 0.39	IVIG+AB	105
n.r	0.45, 0.22	AB	30
Yes	0.17, 0.10	AB	42
No	0.38, 0.38	IVIG	n.r
n.r.	n.r, 0.38	none	162

Familial cases	First- and last serum IgM (g/l)	Treatment	Follow-up period (months)
No	0.10, 0.10	None	38
No	0.14, 0.12	None	70
No	0.20, 0.37	AB	67
No	0.10, 0.10	None	39
No	0.16, 0.14	None	101

Table 4.2. Continued.

B. Lymphocyte subsets in adults with true (n=5) or possible sIgMdef (n=4).										
Patient	**CD3+ T cells**		**CD4+ T cells**		**CD8+ T cells**		**CD19+ B cells**		**CD3-CD16+ CD56+ NK cells**	
	x 10^e9/l	%	x 10^e9/l	%	x 10^e9/l	%	x 10^e9/l	%	x 10^e9/l	%
True sIgMdef										
A1	0.9		0.6		0.3		0.2		0.12	
A2	1.3		0.9		0.4		0.4		0.68	
A3	2.0		1.5		0.6		0.1		0.20	
A4	1.7		1.0		0.6		0.6		0.22	
A5	0.8		0.5		0.3		0.3		0.19	
Possible sIgMdef										
A7		70		39		27		13		13
A10	2.0		0.9		1.0		0.2		0.10	
A12		79		47		29		10		10
A13	1.3		0.9		0.4		0.1		0.12	

Table 4.2. [a]Age at first sample collection.
Abbreviations: A, adult; AB, prophylactic antibiotics; F, female; IgM, immunoglobulin M; M, male; sIgMdef, selective IgM deficiency. Reference ranges from: Schatorjé et al. Scand J Immunol 2011;74(5):502-10[35].

Comparison between the tertiary and secondary centre cohorts of adult patients

We first compared the 13 adults with true or possible sIgMdef from this tertiary centre cohort with the 42 adults with true or possible sIgMdef from the secondary centre cohort we previously published [15]. These two cohorts differ in the type of population from which the data were collected (general hospital versus specialized medical centres) and in the way of collecting the data (analysing all laboratory data with decreased serum IgM versus only analysing patients reported as diagnosed with IgM deficiency by an immunologist). Given this different patient selection process, further immunological analyses were as expected more often performed in the tertiary centre cohort: repeated measurements of serum IgM in 86% vs 14% (Fisher exact test, $p < 0.001$), measurements of IgG subclasses in 92% vs 14% (Fisher exact test, $p < 0.001$), and pneumococcal vaccination responses in 42% vs 7% (Fisher exact test, $p = 0.003$). Not only in the previously described secondary centre cohort, but also in this tertiary centre cohort but few patients can be classified as *true* sIgMdef (**Figure 4.4**).

In contrast to the tertiary centre cohort, adults in the secondary centre cohort were often asymptomatic. First serum IgM levels were significantly higher in the secondary centre cohort (mean 0.30 g/l, 95%CI 0.28-0.33) compared to the tertiary centre cohort (mean 0.27 g/l, 95%CI 0.17-0.37, $p = 0.01$; **Figure 4.5A**).

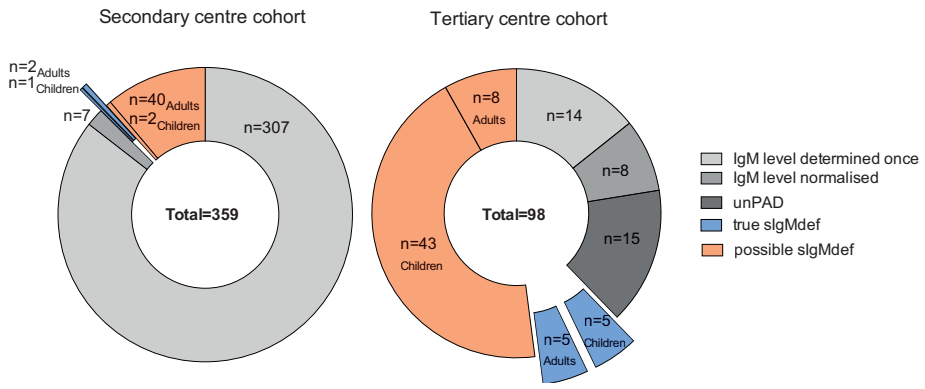


Figure 4.4. Classification of patients with decreased serum IgM in the tertiary (n=98) and secondary (n=359) centre cohorts.

Figure 4.4. Abbreviations: sIgMdef, selective IgM deficiency; unPAD, unclassified primary antibody deficiency.

Second, comparisons were made between three groups: 1) symptomatic adults from the tertiary centres (n=13), 2) symptomatic adults from the secondary centre (n=18), and 3) asymptomatic adults from the secondary centre (n=24) (**Table 4.3**). The mean age at diagnosis was significantly higher in patients without symptoms that could be related to antibody deficiency (mean 65 years, 95%CI 60-70) compared to those with symptoms from the secondary centre (mean 56 years, 95%CI 49-64) and tertiary centres (mean 40 years, 95%CI 31-49; $p<0.01$). We evaluated the mean first serum IgM levels in the different clinical manifestations (**Figure 4.5B**). Two symptoms, autoimmunity and fatigue, showed a significant difference, the patients with the symptoms having lower IgM levels (autoimmunity n = 6, mean 0.21 g/l, 95%CI 0.09-0.33; no autoimmunity n=49, mean 0.30 g/l, 95%CI 0.27-0.33; $t(53) = -2.137$, $p=0.037$; fatigue n = 9, mean 0.22 g/l, 95%CI 0.16-0.29; no fatigue n=46, mean 0.31 g/l, 95%CI 0.27-0.34; $t(53) = -2.265$, $p=0.03$). When combining all symptoms that could be related to antibody deficiency, adults with these symptoms (n=31) had significantly lower IgM levels compared to adults without these symptoms (n=24) (mean 0.27 g/l, 95%CI 0.22-0.31 versus mean 0.33 g/l, 95%CI 0.30-0.36; $t(47.094) = 2.353$, $p=0.02$, **Figure 4.5C**).

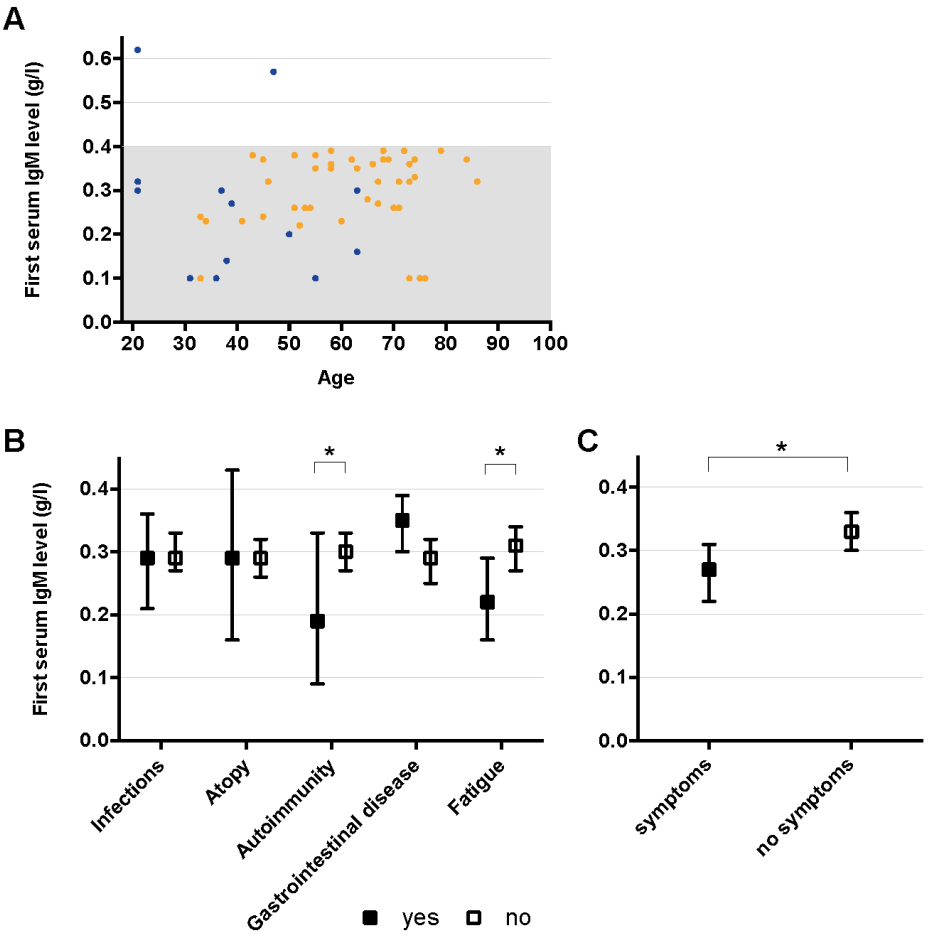


Figure 4.5. First serum IgM levels in the adults from the tertiary and secondary centre cohorts.

*Figure 4.5. Tertiary centre cohort n=13, blue; secondary centre cohort n=42, yellow. First serum IgM levels (y-axis) and age at the date of first serum sample (x-axis) (A). The grey area in the graph represents decreased IgM levels according to the ESID diagnostic protocol values [18]. Mean first serum IgM levels + 95%CI (g/l) in the different clinical manifestations of adults from both tertiary and secondary centres (B), and in those with (n=30) and without (n=25) symptoms that could be related to antibody deficiency (C). *Two-sided t-test; p< 0.05.*

Table 4.3. Clinical and laboratory features of the adults with true or possible sIgMdef. Tertiary centre cohort (n=13), and symptomatic (n=18) and asymptomatic (n=24) secondary centre cohort.

	Tertiary centre symptomatic (n=13)	**Secondary centre** symptomatic (n=18)	**Secondary centre** asymptomatic[a] (n=24)	**p value**
Age[b], years (95% CI)	40 (31-49)	56 (49-64)	65 (60-70)	**<0.01**[*]
Males, n (%)	7 (54)	11 (61)	12 (50)	0.79[#]
Follow-up period, months (95% CI)	64 (36-92)	68 (52-84)	80 (65-95)	0.41[*]
Clinical manifestation(s), n (%)				
Infectious manifestations	7 (54)	9 (50)	0 (0)	**<0.01**[#]
Atopic manifestations	2 (15)	5 (28)	0 (0)	**0.02**[#]
Autoimmune manifestation	3 (23)	1 (6)	0 (0)	**0.05**[#]
Gastrointestinal disease	2 (15)	2 (11)	3 (12)	1.00[#]
Long-lasting fatigue	3 (23)	5 (28)	1 (4)	0.09[#]
First IG levels, g/l (95% CI)				
Serum IgM	0.27 (0.17 – 0.37)	0.27 (0.22-0.31)	0.33 (0.30-0.36)	0.11[*]
Serum IgG	12.1 (11.5-13.6)	10.5 (9.5-11.4)	10.7 (9.9-11.5)	0.09[*]
Serum IgA	2.4 (1.8-3.0)	2.7 (1.9-3.5)	2.9 (2.2-3.6)	0.63[*]
Treatment, n (%)[c]				
Prophylactic antibiotics	3 (23)	0 (0)	0(0)	**0.01**[#]

Table 4.3. [a] This means no symptoms potentially related to antibody deficiency were present. [b] Age at first sample collection. [c] None of the adults were treated with immunoglobulins.

[*] ANOVA. [#] Fisher exact test.

Abbreviations: CI, confidence interval; IG, immunoglobulin.

DISCUSSION

When isolated decreased serum IgM levels are repeatedly found in a patient, clinicians are confronted with a dilemma. To date, it is not clear what the clinical consequences of such a finding are, and whether and if so how such patients should be treated. The results of our study underline these challenges. Not only in our previously published secondary centre cohort [15], but also in this tertiary centre cohort as well as in other cohorts in the literature [4,5,13,14,16] only few patients with decreased serum IgM levels have *true* sIgMdef. This condition is probably very rare.

However, the adults with more severely decreased serum IgM levels were more likely to be younger and to be symptomatic. This information can help in interpreting the clinical significance when an isolated decreased serum IgM level is discovered. While just below normal values tend to have little clinical meaning, we suggest that lower cut-off values than the current 'two standard deviations (SD) below the mean' probably distinguish the clinically relevant category of patients. We propose to develop a classification for sIgMdef similar to the one previously developed for selective IgA deficiency. This classification distinguishes selective IgA deficiency (serum IgA <0.07g/l) from the often clinically irrelevant partial IgA deficiency (serum IgA >0.07g/l but 2 SD below normal age-adjusted means) [20,21]. For selective IgM deficiency, such a cut-off value will have to be determined in future studies.

Our study has several limitations. First, our results are based on a still relatively small cohort including not only true but also possible sIgMdef. This group contained a high number of children, which is in contrast to few children reported in the literature [13]. This is probably bias resulting from the type of centres that decided to participate in the study. Second, it is possible that mildly affected patients with a known genetic defect are 'hidden' in the sIgMdef population and fulfil the criteria for syndromic immunodeficiencies instead [22–25]. This can only be revealed by genetic testing in such cases. Third, age-matched cut-off values varied widely between the centres; when using the ESID diagnostic protocol values, even fewer patients had true sIgMdef (1 child, 5 adults). This cannot only be explained by variations in technique or in genetic, ethnic or geographical differences, which have also been shown to influence serum IgM levels [26–32]. Almost all centres (14 out of 15) used immunonephelometric or immunoturbidimetric techniques, which have been demonstrated to be reliable and to have good comparability [33,34]. Although inter-laboratory variability in the current methodologies can make unification of reference values challenging, investigating opportunities for achieving this would be worthwhile.

In conclusion, even this multi-centre study could not solve the dilemma. Even enlarging the study to global proportions will probably not answer our questions. To be able to explore the clinical consequences of *true* sIgMdef, full analysis and accurate description of all patients in whom a decreased serum IgM is found would be more effective, leaving no patients with *possible* sIgMdef to dilute the results.

REFERENCES

1. Louis AG, Gupta S. Primary selective IgM deficiency: an ignored immunodeficiency. Clin Rev Allergy Immunol. 2014 Apr;46(2):104–11.
2. Entezari N, Adab Z, Zeydi M, Saghafi S, Jamali M, Kardar GA, et al. The prevalence of Selective Immunoglobulin M Deficiency (SIgMD) in Iranian volunteer blood donors. Hum Immunol. 2016 Jan;77(1):7–11.
3. Guill MF, Brown DA, Ochs HD, Pyun KH, Moffitt JE. IgM deficiency: clinical spectrum and immunologic assessment. Ann Allergy. 1989 Jun;62(6):547–52.
4. Yel L, Ramanuja S, Gupta S. Clinical and immunological features in IgM deficiency. Int Arch Allergy Immunol. 2009;150(3):291–8.
5. Louis AG, Agrawal S, Gupta S. Analysis of subsets of B cells, Breg, CD4Treg and CD8Treg cells in adult patients with primary selective IgM deficiency. Am J Clin Exp Immunol. 2016;5(1):21–32.
6. Hobbs JR, Milner RD, Watt PJ. Gamma-M deficiency predisposing to meningococcal septicaemia. Br Med J. 1967 Dec;4(5579):583–6.
7. Kaufman HS, Hobbs JR. Immunoglobulin deficiencies in an atopic population. Lancet (London, England). 1970 Nov;2(7682):1061–3.
8. Silver HK, Shuster J, Gold P, Freedman SO. Leukopenia, leukoagglutinins, and low IgM in a family with severe febrile illnesses. Clin Immunol Immunopathol. 1973 Jan;1(2):220–9.
9. Inoue T, Okumura Y, Shirama M, Ishibashi H, Kashiwagi S, Okubo H. Selective partial IgM deficiency: functional assessment of T and B lymphocytes in vitro. J Clin Immunol. 1986 Mar;6(2):130–5.
10. Ohno T, Inaba M, Kuribayashi K, Masuda T, Kanoh T, Uchino H. Selective IgM deficiency in adults: phenotypically and functionally altered profiles of peripheral blood lymphocytes. Clin Exp Immunol. 1987 Jun;68(3):630–7.
11. Yamasaki T. Selective IgM deficiency: functional assessment of peripheral blood lymphocytes in vitro. Intern Med. 1992 Jul;31(7):866–70.
12. Cipe FE, Dogu F, Guloglu D, Aytekin C, Polat M, Biyikli Z, et al. B-cell subsets in patients with transient hypogammaglobulinemia of infancy, partial IgA deficiency, and selective IgM deficiency. J Investig Allergol Clin Immunol. 2013;23(2):94–100.
13. Goldstein MF, Goldstein AL, Dunsky EH, Dvorin DJ, Belecanech GA, Shamir K. Pediatric selective IgM immunodeficiency. Clin Dev Immunol. 2008;2008:624850.
14. Goldstein MF, Goldstein AL, Dunsky EH, Dvorin DJ, Belecanech GA, Shamir K. Selective IgM immunodeficiency: retrospective analysis of 36 adult patients with review of the literature. Ann Allergy Asthma Immunol. 2006 Dec;97(6):717–30.
15. Janssen LMA, Macken T, Creemers MCW, Pruijt JFM, Eijk JJJ, De Vries E. Truly selective primary IgM deficiency is probably very rare.
16. Chovancova Z, Kralickova P, Pejchalova A, Bloomfield M, Nechvatalova J, Vlkova M, et al. Selective IgM Deficiency: Clinical and Laboratory Features of 17 Patients and a Review of the Literature. J Clin Immunol. 2017 Aug;37(6):559–74.
17. Hassanein HA, Elbadry MI. Selective immunoglobulin M deficiency in an adult with miliary tuberculosis: A clinically interesting coexistence. A case report and review of the literature. Int J mycobacteriology. 2016 Mar;5(1):106–10.
18. Guzman D, Veit D, Knerr V, Kindle G, Gathmann B, Eades-Perner AM, et al. The ESID Online Database network. Bioinformatics. 2007 Mar;23(5):654–5.

19. De Vries E. Patient-centred screening for primary immunodeficiency: A multi-stage diagnostic protocol designed for non-immunologists. Clin Exp Immunol. 2006;
20. Yel L. Selective IgA deficiency. J Clin Immunol. 2010 Jan;30(1):10–6.
21. Al-Herz W, Bousfiha A, Casanova J-L, Chapel H, Conley ME, Cunningham-Rundles C, et al. Primary immunodeficiency diseases: an update on the classification from the international union of immunological societies expert committee for primary immunodeficiency. Front Immunol. 2011;2:54.
22. Haddad ZH, Allen RF, Towner JW, Wilson MG. IgA, IgM, and partial deletion of chromosome 18. Lancet (London, England). 1969 Mar;1(7596):678.
23. Ostergaard PA. A girl with recurrent infections, low IgM and an abnormal chromosome number 1. Acta Paediatr Scand. 1973 Mar;62(2):211–5.
24. Kung S-J, Gripp KW, Stephan MJ, Fairchok MP, McGeady SJ. Selective IgM deficiency and 22q11.2 deletion syndrome. Ann Allergy Asthma Immunol. 2007 Jul;99(1):87–92.
25. Al-Herz W, McGeady SJ, Gripp KW. 22q11.2 deletion syndrome and selective IgM deficiency: an association of a common chromosomal abnormality with a rare immunodeficiency. Vol. 127A, American journal of medical genetics. Part A. United States; 2004. p. 99–100.
26. Ambrosino DM, Black CM, Plikaytis BD, Reimer CB, Lee MC, Evatt BL, et al. Immunoglobulin G subclass values in healthy black and white children. J Pediatr. 1991 Dec;119(6):875–9.
27. Yang M, Wu Y, Lu Y, Liu C, Sun J, Liao M, et al. Genome-wide scan identifies variant in TNFSF13 associated with serum IgM in a healthy Chinese male population. PLoS One. 2012;7(10):e47990.
28. Gonzalez-Quintela A, Alende R, Gude F, Campos J, Rey J, Meijide LM, et al. Serum levels of immunoglobulins (IgG, IgA, IgM) in a general adult population and their relationship with alcohol consumption, smoking and common metabolic abnormalities. Clin Exp Immunol. 2008 Jan;151(1):42–50.
29. Aksu G, Genel F, Koturoglu G, Kurugol Z, Kutukculer N. Serum immunoglobulin (IgG, IgM, IgA) and IgG subclass concentrations in healthy children: a study using nephelometric technique. Turk J Pediatr. 2006;48(1):19–24.
30. Kacprazak-Bergman I. Sexual dimorphism of heritability of immunoglobulin levels. Ann Hum Biol. 1994;21(6):563–9.
31. Kohler PF, Rivera VJ, Eckert ED, Bouchard TJJ, Heston LL. Genetic regulation of immunoglobulin and specific antibody levels in twins reared apart. J Clin Invest. 1985 Mar;75(3):883–8.
32. Siegel M, Lee SL, Ginsberg V, Schultz F, Wong W. Racial differences in serum gamma globulin levels: comparative data for Negroes, Puerto Ricans, and other Caucasians. J Lab Clin Med. 1965 Nov;66(5):715–20.
33. Mali B, Armbruster D, Serediak E, Ottenbreit T. Comparison of immunoturbidimetric and immunonephelometric assays for specific proteins. Clin Biochem. 2009 Oct;42(15):1568–71.
34. Denham E, Mohn B, Tucker L, Lun A, Cleave P, Boswell DR. Evaluation of immunoturbidimetric specific protein methods using the Architect ci8200: comparison with immunonephelometry. Ann Clin Biochem. 2007 Nov;44(Pt 6):529–36.
35. Schatorje EJH, Gemen EFA, Driessen GJA, Leuvenink J, van Hout RWNM, van der Burg M, et al. Age-matched reference values for B-lymphocyte subpopulations and CVID classifications in children. Scand J Immunol. 2011 Nov;74(5):502–10.

Supplementary Table 4.1.

A. Clinical manifestations of paediatric patients with *true* (n=5) or *possible* (n=43) selective primary IgM deficiency (sIgMdef).

Patient	Age Years/ gender	Clinical manifestation(s)	Acute respiratory infection
ESID criteria completely fulfilled (*true* sIgMdef)			
1	0/M	Recurrent pneumonia	X
2	1/M	Recurrent ENT infections	X
3	4/F	Complicated atypical mycobacterial adenitis	X
4	4/F	Atopic dermatitis, eczema, food allergy, asthma, warts	X
5	11/F	Severe eczema	
Data on IgG subclasses present, but no data on pneumococcal antibody responses (*possible* sIgMdef)			
6	4/F	Recurrent otitis	
7	7/M	Upper respiratory tract infections	
8	9/M	Biliary colic	
9	5/M	Complicated pneumonia	X
10	5/F	Skin infections, bronchiectasis	X
11	9/M	Recurrent fever	
12	13/M		
13	5/F	Recurrent respiratory infections with bilateral otitis	
14	2/M		X
15	3/M		
16	8/M		
17	5/M		X
18	10/M		
19	8/M		
20	7/M		
21	3/M		X
22	14/M		
23	15/M		
24	10/M		
25	6/M		X
26	13/M		
27	4/M		X
28	6/M		
29	15/M		X

	Recurrent respiratory infections	Proven pneumonia	Atopy	Auto-immunity	Gastro-intestinal disease	Fatigue	No symptoms
	X	X					
	X	X					
	X	X	X			X	
			X		X		
			X				
	X		X		X		
	X						
					X	X	
	X	X	X				
	X						
	X						
	X						
	X	X					
	X	X					
	X		X				
					X		
	X						
	X						
	X						
	X						
	X		X				
					X		
	X						
	X		X				
	X		X				
	X						
	X						
	X						

Supplementary Table 4.1. Continued.

A. Clinical manifestations of paediatric patients with *true* (n=5) or *possible* (n=43) selective primary IgM deficiency (sIgMdef).			
Patient	**Age Years/ gender**	**Clinical manifestation(s)**	**Acute respiratory infection**
No data on pneumococcal antibody responses and no data on IgG subclasses (*possible* sIgMdef)			
30	0/M		
31	0/M		
32	1/M		X
33	1/M		X
34	4/M	Bilateral pneumonia with an abscess in the left pulmonary lobe	X
35	4/M		X
36	5/F	Sinusitis	X
37	5/M		X
38	6/M	Sinusitis	
39	6/M		
40	6/F		
41	6/F		X
42	8/M	Sinusitis, bronchitis	
43	10/F	Glomerulopathy	
44	10/M	Aphthous mouth ulcers	
45	11/M	Skin infections	
46	11/M	Pharyngotonsillitis	
47	12/M		
48	17/M		

	Recurrent respiratory infections	Proven pneumonia	Atopy	Auto-immunity	Gastro-intestinal disease	Fatigue	No symptoms
	X						
							X
		X					
	X						
	X						
				X			
					X		
	X						
	X						
	X						
	X		X				
	X						

Supplementary Table 4.1. Continued.

B. Clinical manifestations and characteristics of adult patients with *true* (n=5) or *possible* (n=8) selective primary IgM deficiency (sIgMdef).			
Patient	**Age Years/ gender**	**Clinical manifestation(s)**	**Acute respiratory infection**
ESID criteria completely fulfilled (*true* sIgMdef)			
1	36/F	Atopic dermatitis, allergic rhinitis, sinusitis	
2	38/F	Bronchitis, nasopharyngitis, chronic hepatitis B	
3	50/F	Bronchiectasis, celiac disease	
4	55/M	Vertebral pain syndrome	
5	63/F	Sjogren's syndrome, alopecia, multiple lung cysts	
Data on IgG subclasses present, but no data on pneumococcal antibody responses (*possible* sIgMdef)			
6	21/M		
7	21/F		
8	21/M	Meningococcal sepsis	
9	31/M	Arthralgia	
10	37/M	Recurrent sinus and chest infections	
11	39/F		
12	47/M		X
Data on pneumococcal antibody responses present, but no data on IgG subclasses (*possible* sIgMdef)			
13	63/M	Recurrent HSV encephalitis, tinnitus	

Supplementary Table 4.1. Abbreviations: ENT=ear-nose-troat; F=female; HSV=Herpes Simplex virus; M=male; sIgMdef = selective primary IgM deficiency.

	Recurrent respiratory infections	Proven pneumonia	Atopy	Auto-immunity	Gastro-intestinal disease	Fatigue	No symptoms
	X		X				
	X						
	X			X		X	
						X	
				X		X	
	X						
	X						
						X	
	X			X	X		
					X		
			X				

Supplementary Table 4.2. Clinical and laboratory features of children with true or possible sIgMdef. Turkish cohort (n=24), and non-Turkish cohort (n=24).

	Turkey (n=24)	**Other countries** (n=24)	**p value**
Age[a], years	7.3 (SD 5.0)	6.4 (SD 3.5)	0.45*
Males, n (%)	22 (92)	16 (67)	0.07#
Follow-up period, months	49 (SD 31)	62 (SD 51)	0.37*
Clinical manifestation(s), n (%)			
Infectious manifestations	20 (83)	18 (75)	0.72#
Atopic manifestations	4 (17)	4 (17)	1.00#
Autoimmune manifestation	0 (0)	0 (0)	-
Gastrointestinal disease	2 (8)	2 (8)	1.00#
Long-lasting fatigue	0 (0)	1 (4)	1.00#
First IG levels, g/l			
Serum IgM	0.40 (SD 0.10)	0.30 (SD 0.12)	**0.01***
Serum IgG	10.2 (SD 2.8)	8.8 (SD 3.1)	0.11*
Serum IgA	1.68 (SD 1.18)	1.12 (SD 0.69)	0.05*
Treatment, n (%)			
Prophylactic antibiotics	5 (21)	5 (21)	1.00#
IVIG	0 (0)	3 (13)	0.23#

*Supplementary Table 4.2. [a]Age at first sample collection. *Two-sided t-test. #Fisher exact test. Abbreviations: IG, immunoglobulin; IVIG, intravenous immunoglobulins; SD, standard deviation.*

Chapter 5

Lessons learned from the clinical presentation of common variable immunodeficiency disorders: a systematic review and meta-analysis

Lisanne MA Janssen, MD, Michiel van der Flier MD, PhD, Esther de Vries, MD, PhD

Front Immunol. 2021; 12:620709

ABSTRACT

Background

Diagnostic delay in common variable immunodeficiency disorders (CVID) is considerable. There is no generally accepted symptom-recognition framework for its early detection.

Objective

To systematically review all existing data on the clinical presentation of CVID.

Methods

PubMed, EMBASE and Cochrane were searched for cohort studies, published January/1999-December/2019, detailing the clinical manifestations before, at and after the CVID-diagnosis.

Results

In 51 studies (n=8521 patients) 134 presenting and 270 total clinical manifestations were identified. Recurrent upper and/or lower respiratory infections were present at diagnosis in 75%. Many patients had suffered severe bacterial infections (osteomyelitis 4%, meningitis 6%, septicaemia 8%, mastoiditis 8%). Bronchiectasis (28%), lymphadenopathy (27%), splenomegaly (13%), inflammatory bowel disease (11%), autoimmune cytopenia (10%) and idiopathic thrombocytopenia (6%) were also frequently reported. A bimodal sex distribution was found, with male predominance in children (62%) and female predominance in adults (58%). 25% of CVID-patients developed other manifestations besides infections in childhood, this percentage was much higher in adults (62%). Immune-dysregulation features, such as granulomatous-lymphocytic interstitial lung disease and inflammatory bowel disease, were more prominent in adults.

Conclusions

The shift from male predominance in childhood to female predominance in adults suggests differences in genetic and environmental aetiology in CVID and has consequences for pathophysiologic studies. We confirm the high frequency of respiratory infections at presentation, but also show a high incidence of severe bacterial infections such as sepsis and meningitis, and immune dysregulation features including lymphoproliferative, gastrointestinal and autoimmune manifestations. Early detection of CVID may be improved by screening for antibody deficiency in patients with these manifestations.

INTRODUCTION

Common variable immunodeficiency disorders (CVID) is a collection of heterogeneous clinical manifestations linked by low serum levels of immunoglobulins and primary failure of specific antibody production [1–3]. The rates of serious comorbidities and resulting mortality of patients with CVID drastically exceed the respective rates in the general population, imposing a high disease burden to the individual patient [4,5]. Although CVID is the most common symptomatic primary immunodeficiency (PID), it is still a rare disease with a greatly varying observed prevalence between countries, ranging in "industrialized countries" from 6.9/100,000 in Finland to 0.6/100,000 in Spain [6–14] and even lower observed prevalence rates (<0.5/100,000) in "developing" countries [15]. Therefore, CVID has a low prevalence in primary care and general hospital settings, where non-immunologists have little knowledge of this disease. Also, respiratory infections and non-infectious complications of CVID such as lymphoproliferation, granulomatous disease and autoimmunity are much more prevalent *without* concomitant CVID. This makes it challenging to front-line clinicians to recognize CVID in these cases. Because of the variability of presenting clinical manifestations, patients visit various physicians of different specialties in search of a diagnosis, which increases the risk of missing the overarching clinical pattern and thereby overlooking the underlying hypogammaglobulinemia [16].

Timely diagnosis and optimal management are likely to result in improved clinical and quality-of-life outcomes for patients with CVID, higher participation in society (school, work) and lower health care costs [4,17–19]. Reducing diagnostic delay is therefore crucial; current approaches mainly comprise improving education and awareness of clinicians in both primary and secondary care. Already a long time ago, the Jeffrey Modell Foundation (JMF) developed ten (mainly paediatric) [20] and the European Society for Immunodeficiencies (ESID) six (adult) 'Warnings Signs' to indicate PIDs [21]. Unfortunately, these signs have turned out to have a low sensitivity for timely PID diagnosis [22,23].

In order to improve our insight in the early presentation of CVID and to assist physicians in its timely detection, we aimed to systematically identify and collate existing published cohort studies on the presenting clinical manifestations at and before diagnosis. In addition, we included the overall clinical manifestations during disease follow-up in our systematic review; this was done separately for children and adults to evaluate age-related differences and similarities in paediatric and adult onset CVID.

MATERIALS AND METHOD

Search strategy

We searched EMBASE, Cochrane and PubMed from January 1999 to December 2019 (inclusive) using a combination of subject headings and free text incorporating the terms 'common variable immunodeficiency', 'late onset hypogammaglobulinemia', and 'diagnosis', and limited to English language and humans. Reference lists of included studies were also searched for potentially relevant studies (snowball method). The complete search strategy is detailed in the **Supplementary appendix eSearch**. The protocol of this systematic review has been registered on PROSPERO with registration number CRD42019121384.

Study selection

We considered all primary research studies for selection, either retrospective or prospective, of any study design (e.g., case series, cohort), describing the clinical manifestations for a minimum of 10 patients with CVID. Two researchers (LJ and EdV) independently screened titles and abstracts of all papers, excluding clearly irrelevant studies. Hereafter, they independently reviewed the full text of remaining papers to assess eligibility. If multiple updates of a cohort were published, the most recent study with the largest dataset describing the total clinical picture of their CVID cohort was included, in order to avoid duplicates of patients in our review. The large European multicentre study by Gathmann et al. [24] was excluded for analysis to avoid overlapping data, because this study collated data from multiple centres that already published a substantial amount of their data as single-centre cohorts in more detail. Three European multicentre studies [25–27] partially overlapped in their included centres; in this case the largest multicentre study by Chapel et al. describing the overall clinical picture of CVID was included [26] (for details about the handling of overlapping data, see **Supplementary Table 5.1**). Studies that selected cases based on the presence of only certain clinical features of CVID (e.g., only granulomatous, pulmonary, gastrointestinal or autoimmune manifestations) were excluded to avoid giving disproportionate weight to those features in the data synthesis, unless the total number of CVID patients from which these cases were selected was also reported. When the same centre/registry published an article about their total cohort and another article in which children and/or adults were separately described, these children- and adult-specific overlapping data were only included in the subgroup analysis for children vs adults. Any uncertainties regarding study selection were discussed between LJ, MvdF, and EdV.

Quality assessment

After assembling a shortlist of studies eligible for potential inclusion, LJ assessed the risk of bias in these studies to ensure that only those studies with an acceptable risk of bias were included. This quality assessment was checked by EdV. Because there is no validated quality checklist for assessing retrospective cohort studies, we constructed a checklist based on

relevant items from the MOOSE (meta-analysis of observational studies in epidemiology) reporting guideline for observational studies [28], the STROBE (strengthening the reporting of observational studies in epidemiology) reporting guideline for cohort studies [29] and CASP (critical appraisal skills program) guidelines for case-control and cohort studies (checklist in **Supplementary Table 5.2**) [30]. Quality was assessed as 'acceptable' or 'unacceptable' in three domains: definition of CVID, selection of cases, and methods for extracting data on included cases. 'Acceptable' for case definition required cases to be defined according to the diagnostic criteria of ESID/Pan-American Group for Immunodeficiency (PAGID) [3], the ESID Registry working definitions for clinical diagnosis of PID (www.esid.org), the International Union of Immunological Societies (IUIS) criteria [31], the World Health Organization (WHO) scientific group [32] or the international consensus document (ICON) [33] (**Supplementary Table 5.3**), or - if no reference was made to which diagnostic criteria were used - description of the inclusion criteria corresponding to the above described diagnostic criteria. Although we only included articles about paediatric CVID that reported to have only included established CVID patients, we cannot completely rule out that a few of these patients actually had transient hypogammaglobulinemia of infancy. 'Acceptable' for case selection required that at least two of the participants' baseline characteristics were clearly documented and that the characteristics of cases were sufficiently consistent with the current knowledge regarding CVID (i.e., the age and sex distribution of cases matched the known epidemiology of CVID). 'Acceptable' for data extraction required the use of a standardized data collection format and/or the objective measurement of signs (e.g., CT confirmation of bronchiectasis, biopsy confirmation of granulomas). Disagreements between the two reviewers (LJ and EdV) were discussed with a third reviewer (MvdF) until agreement was achieved. Only studies considered by the two reviewers to be acceptable for case definition and to pass in at least one other domain were included, and data related to the 'unacceptable' domain were not included in this review.

Data extraction

Data were extracted from included studies by LJ using a standardized Microsoft Excel spread sheet and were checked by EdV and MvdF. We extracted study characteristics including year of publication, country, recruitment periods, number and type of centres, study design, number of patients, age and sex. Clinical manifestations were recorded, and numbers of patients with each manifestation were noted. This was done separately for the clinical manifestations at presentation (at or before diagnosis) and overall (at any timepoint). When this distinction was not described, the manifestations were collected as 'overall' in the standardized format. When a clinical manifestation was not discussed in a study, we made no assumption about whether or not that manifestation had occurred in that population but recorded this item as 'missing data' for the respective study in the standardized format. We deliberately chose to use the exact wordings of the included studies, to avoid interpretation

bias. Clinical manifestations were never counted twice. For example, where one study separately described sinusitis and otitis, another study only mentioned 'upper respiratory tract infections'. In addition, we recorded whether in a cohort children, adults or both were described. A paediatric cohort was defined as age during follow-up <18 years old, which was comparable to the cut-off value for children's age provided by the original studies. We did not contact the authors of included papers to collect additional information.

Statistical analysis

We used MetaXL (version 5.3, EpiGear International, Queensland, Australia) to calculate proportions and standard errors (SEs) of proportions for each clinical manifestation in each included study [34]. To combine the results of multiple cohorts, we calculated pooled proportions of each clinical manifestation using the metan command. Anticipating high heterogeneity between included studies, we performed random effects meta-analysis using the DerSimonian and Laird method and standard methods to calculate I^2 as an estimate of heterogeneity. In addition, we conducted two subgroup analyses using the same techniques: 1) children vs adults, and 2) clinical manifestations at presentation vs overall clinical manifestations during the disease course. A subgroup analysis based on age was conducted because the few available studies on differences between paediatric-onset and adult-onset CVID have yielded limited and conflicting data [35–37]. In order to improve our understanding of the early presentation of CVID, which can assist clinicians in timely detection of this condition, we focused our analysis on clinical manifestations at or prior to diagnosis.

RESULTS

Search results

After removal of duplicates, we identified 1604 papers. We excluded 1453 after screening titles and abstracts, and a further 96 after full-text assessment (**Figure 5.1**), based on the inclusion criteria (see Method). Reference lists of included studies yielded 21 additional eligible studies. There was full consensus between the authors regarding study inclusion.

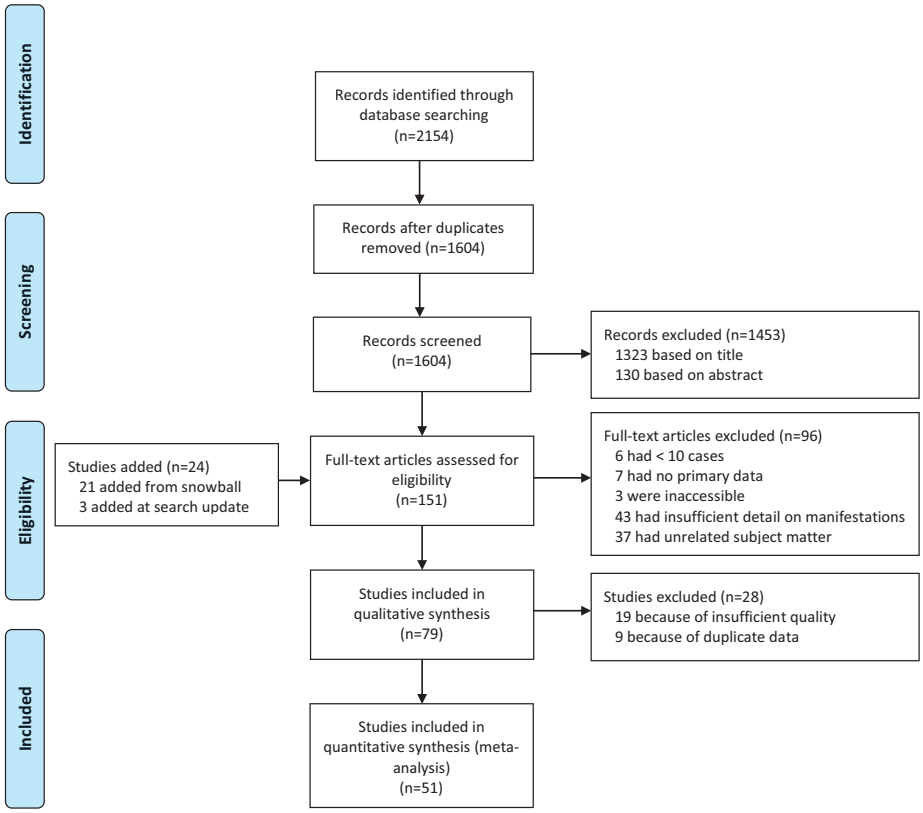


Figure 5.1. Flow chart showing the study selection process.

Characteristics of included studies

The 51 included studies described clinical manifestations in a total of 8521 patients (**Table 5.1**) [5,6,41–50,19,51–60,26,61–70,35,71–80,36,81,37–40]. 50 studies were conducted in one or more centres in one country only, in 18 different countries in total; 1 study included multiple centres from different countries [26]. Most were cohort studies (5 prospective, 42 retrospective) and three compared cases with controls. All 51 studies

extracted data from written/typed hospital records. The majority of studies (n=39) identified cases from hospital records alone; others also used regional, national, or continental registries of primary immunodeficiencies (n=12). Three studies also obtained data from a patient and/or parent-completed questionnaire [56,75,77]. Fifteen studies reported clinical manifestations of their total CVID cohort, but reported in more detail on patients based on the presence of only certain clinical features of CVID: asthma and allergic diseases [46,80], autoimmune manifestations [47,52,54], gastric cancer [71], gastrointestinal manifestations [56,59], granulomatous manifestations [41,48], or pulmonary manifestations [45,62,63,75,76].

Risk of bias of included studies

Most included studies defined cases using the diagnostic criteria of PAGID and ESID (27 studies); other used criteria were: the ESID Registry working diagnosis criteria (8 studies), International consensus document (3 studies), IUIS criteria (2 studies), and WHO classification (4 studies). Seven studies did not report which criteria were used but did describe a CVID diagnosis that corresponded to the above approved classifications. One study reported to use both the diagnostic criteria of PAGID/ESID and the WHO classification [49]. Lack of routine B and T cell immunophenotyping in most studies prohibited an accurate assessment of potential late-onset combined immunodeficiency (LOCID). 37 studies (73%) included all consecutive cases within the study period, with a further 11 studies (22%) describing why a proportion of potentially eligible cases were excluded. In the remaining 3 studies (6%), the proportion of consecutively included cases was unclear.

A weakness of the included studies was lack of clarity at which point in the diagnostic and follow-up pathway clinical features were recorded. Twenty studies explicitly stated when clinical manifestations occurred (at or before diagnosis (n=4), and both at/or before diagnosis and during follow-up (n=16)). The remaining 31 studies were unclear as to when the reported clinical manifestations occurred during the disease course.

Pooled frequencies of clinical characteristics from meta-analysis

Pooled frequencies of demographic information are shown in **Table 5.2**. In paediatric CVID patients, males were in the majority (62%, 95% CI 54-69), while females predominated in the adult CVID patients (58%, 95% CI 53-64). The high pooled proportion of consanguinity in the paediatric and total cohort should be interpreted with caution (31% and 20% respectively; only one study reported this for adults). This proportion varied substantially per country. In an Argentinian cohort none had a history of consanguinity [37], while the rate of consanguinity was very high in an Iranian cohort (72%) [64].

In total, 147 out of a potential of 270 meta-analyses were conducted. For the remaining 123 clinical manifestations, meta-analysis was not possible since the features were each reported in only one study. The high heterogeneity (I^2) statistics in the meta-analyses

(mostly >80%) indicated that the degree of heterogeneity between studies was greater than that expected by chance alone and confirmed the appropriateness of random-effects meta-analysis to generate pooled proportions.

There were 49 specific clinical manifestations for which it was possible to calculate pooled proportions for the subgroup *at presentation*, i.e. 'at or before diagnosis'; these are shown in **Figure 5.2** in comparison with *overall*, i.e. 'at, before or after' diagnosis.

The most frequent clinical manifestations at presentation (reported in ≥39% of patients) are shown above the grey dotted horizontal lines. A history of upper and/or lower respiratory infections was present at diagnosis in three-quarters of patients (upper respiratory tract infections in 73%, lower respiratory tract infections in 73%, sinusitis in 59%, pneumonia in 57%, bronchitis in 57% and otitis in 39%) and severe bacterial infections in 8% (septicaemia), 8% (mastoiditis), 6% (meningitis), and 4% (osteomyelitis).

Bronchiectasis was already present in almost one third of the patients at or before the CVID diagnosis was made (28%, 95% CI 18-40). Non-infectious manifestations that were frequently present at diagnosis were: lymphadenopathy (27%), splenomegaly (13%), inflammatory bowel disease (11%), and autoimmune haematological manifestations (autoimmune cytopenia (10%) and idiopathic thrombocytopenia (6%)). The pooled prevalence's at presentation of urinary tract infection (14%, 95% CI 5-25), thyroid disease (16%, 95% CI 0-43), and diabetes mellitus (2%, 95% CI 0-7) correspond to the estimated lifetime prevalence estimates in the general population of 30% [82], 12% [83], and 0.9% (type 1) [84], respectively. An overview of all reported clinical manifestations at and before diagnosis with – when known – lifetime prevalence estimates from the general population is included in **Supplementary Table 5.4**.

In **Figure 5.3**, all clinical manifestations that were present in ≥10% of patients are shown (as presented, whether or not likely related to CVID); the CVID-associated manifestations are also shown when present in <10% when they were considered important to incorporate by the authors (based on obvious relation with CVID in the current literature or consensus in the field). We grouped these manifestations into eleven distinct clinical categories according to the body system affected and the clinical phenotypes described by Chapel et al. [26]. An overview of all reported clinical manifestations is included in **Supplementary Table 5.5**. Many CVID patients developed non-infectious manifestations during follow-up: bronchiectasis in 32%, lymphadenopathy in 30%, splenomegaly in 29%, polyclonal lymphocytic infiltration in 29%, and autoimmune manifestations in 27%. In addition, a substantial number of patients developed malignancies (10%) and atopic diseases during the entire disease course (asthma (25%), allergic rhinitis (18%)).

Table 5.1. Characteristics of included studies.

Ref	Country	Recruitment period	Source of data	Nr of centres	Nr of pt
Adults					
[44]	USA	1998-2013	Hospital records	1	34
[46]	USA	2008-2018	Hospital records	1	153
[51]	USA	1988-2016 2006-2017	USIDNET registry* Partners$ research patient data registry	- 3	571 205
[37]	Argentina	1997-2008	Hospital records	1	10
[56]	Norway	-	1)Hospital records 2)"GI symptoms" questionnaire	1	104
[58]	Russia	1990-2011	Hospital records	1	57
[62]	The Netherlands	2008-2012	Hospital records	1	47
[68]	France	2004-2007	French DEFI database*	31	252
[5]	USA	1986-2011	Hospital records	1	473
[35]	USA	1988-2016	USIDNET registry*	-	264
[75]	United Kingdom	2014-2015	1)Daily checkbox symptom diaries 2)St George's Respiratory Questionnaire 3)Hospital records	1	134
[45]	USA	1985-2001	Hospital records	1	69
[60]	Brazil	1980-2003	Hospital records	1	71
[70]	France	-	Hospital records	1	57
[36]	USA	2005-2016	Hospital records	1	107
[71]	Italy	2001-2017	Hospital records	3	455
Children					
[39]	Argentina	-	Hospital records	1	28
[49]	Taiwan	1990-2010	Hospital records	1	10
[51]	USA	1988-2016	USIDNET registry*	-	212
[37]	Argentina	1997-2008	Hospital records	1	21
[61]	Spain	1985-2005	Hospital records	1	22
[64]	Iran	1984-2010	Hospital records	1	69
[69]	Poland	1995-2011	Hospital records	1	49
[35]	USA	1988-2016	USIDNET registry*	-	193
[77]	Germany	1990-2004	1)Hospital records 2)Parent/patient-completed data	1	32
[78]	The Netherlands	1995-2008	Hospital records	1	38
[81]	USA	-	Hospital records	1	45
[67]	USA	1992-2005	Hospital records	1	12

Age at diagnosis (years)			Diagnostic delay (years)			Follow-up (years)			Mortality
Median	Mean	Range	Median	Mean	Range	Median	Mean	Total	
-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	4.1%[a]
42	-	-	-	-	-	-	-	-	15.1%
41	-	18-69	-	9.5	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	5	21%
27	-	-	9.5	-	-	9.5	-	-	-
33.9	-	-	6.9	-	0-55	-	-	-	-
-	-	-	-	-	-	-	-	40	19.6%
-	-	18-76.9	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-
-	30.9	-	-	10.9	-	4.5	-	28.3	15.5%
-	-	-	-	-	-	-	-	-	-
-	45	-	-	-	-	-	5.7	-	4%
-	40.1	-	-	-	-	-	11.5	-	17.1%
11.2	11.1	4-16.1	-	5.4	-	-	-	-	-
-	4.5	-	-	1.5	-	-	9.8	-	0%
-	-	-	-	-	-	-	-	-	-
8.5	-	3-17	-	4.5	-	-	-	-	-
7.8	-	2.5-16	-	-	-	-	-	18	-
-	6.76	4-16	-	4.4	-	-	5.2	21	21.7%
8.8	-	2.4-17.3	2.4	-	0-12.2	-	-	-	-
-	-	2-17	-	-	-	-	-	-	-
10.4	-	1.1-17.4	5.8	-	0.2-14.3	-	-	-	-
-	5.5	0.9-12.7	-	-	-	-	-	-	-
-	-	2-16	-	-	-	-	-	-	-
8	8.3	2-17	-	-	-	-	-	-	-

Table 5.1. Continued.

Ref	Country	Recruitment period	Source of data	Nr of centres	Nr of pt
[43]	Turkey	2003-2014	Hospital records	1	28
[36]	USA	2005-2016	Hospital records	1	91
All ages together					
[38]	Iran	1984-2013	Registry database*	14	173
[40]	Turkey	2001-2008	Hospital records	1	23
[42]	Iran	n/a-2014	Hospital records	1	47
[47]	France	2004-2008	French DEFI database*	-	311
[48]	France	-	French DEFI database*	-	436
[26]	Multiple	1996-2006	ESID registry#	7	334
[52]	USA	n/a-2017	USIDNET registry*	50	990
[54]	France	2013-2016	CEREDIH registry*	-	408
[55]	The Netherlands	-	Hospital records	1	32[b]
[57]	Finland	1996-1998	1)Central register 2)Hospital records of 5 university hospitals*	6	95
[63]	Spain	-	Hospital records	1	19
[65]	Iran	1983-2013	Hospital records	1	125
[66]	Turkey	2008-2014	Hospital records	1	31
[72]	Italy	1999-2005	Italian PID Network*	26	224
[73]	Mexico	-	Hospital records*	7	43
[74]	Puerto Rico	-	Hospital records	1	20
[76]	United Kingdom	1997-1998	Hospital records	1	47
[59]	Iran	1997-2004	Iranian PID registry	1	39
[50]	USA	1973-1998	Hospital records	1	248
[41]	USA	-	Hospital records	2	455
[53]	USA	2011-2015	Hospital records	1	128
[19]	Italy	1985-2015	Hospital records	1	75
[6]	Finland	2007-2015	Hospital records$	3	106
[79]	Poland	1990-2017	Internet database$	4	77
[80]	Iran	-	Hospital records	1	187

*Table 5.1. *Nationwide. #Continentwide. $Regionwide. [a]This is the mortality percentage of the total USIDNET cohort (n=884); [b]Two patients with thymoma were excluded (Good syndrome). Abbreviations: CEREDIH, Centre de Référence Déficits Immunitaires Héréditaires; ESID, European society for immunodeficiency; FU, follow-up; GI, gastro-intestinal; Nr, number; PID, primary immunodeficiency; pt, patients; USIDNET, United States Immunodeficiency Network.*

Age at diagnosis (years)			Diagnostic delay (years)			Follow-up (years)			Mortality
Median	Mean	Range	Median	Mean	Range	Median	Mean	Total	
5.9	6.7	1-15	-	-	-	-	-	-	-
-	12	-	-	-	-	-	8.6	-	13%
-	12.3	4-54	4	-	0.25-39	-	-	29	30%
30.5	33	13-73	-	-	1-32	-	-	-	-
-	20.2	-	-	9	-	-	6.8	23	6%
35.2	-	16-58	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-
33	35.3	-	5	7.5	0-61	22.5	25.6	-	14.5%
-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-
34.3	-	0-63	-	-	-	15.8	-	-	2.9%
33	32	0.5-73	5	8.5	0.2-37	-	-	-	4%
-	23.2	-	-	-	-	-	-	-	-
8.3	-	0-54	4	-	0-51	-	-	25	27.2
23	-	-	14	-	-	-	-	-	-
-	26.6	2-73	-	8.9	-	11.5	11.5	34	6%
19	-	-	-	12.5	-	-	-	-	-
-	-	5-30	-	-	-	-	-	-	-
35	-	5-72	4	-	0.8-25	-	-	12	17%
12	16	3-55	-	-	-	-	-	-	-
-	31	3-79	-	-	-	7	-	25	27%
26	-	2-59	-	-	-	-	-	25	20.5
-	-	-	-	-	-	-	-	-	-
40	-	-	7	-	-	9	10.24	30	5.3%
-	-	-	-	-	-	-	-	-	9.4%
-	32.29	-	-	10,13	-	-	4.26	-	-
-	-	-	-	-	-	-	-	-	-

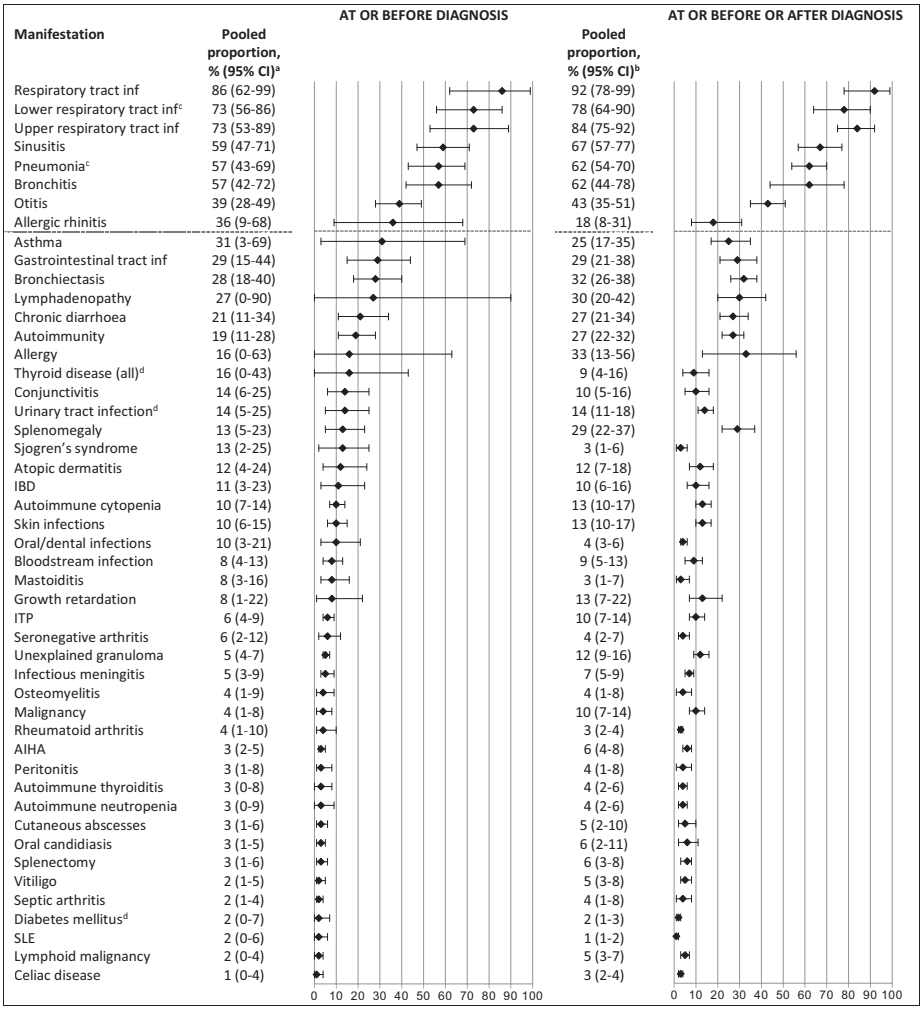


Figure 5.2. Frequency of reported clinical manifestations at presentation vs overall clinical manifestations during the disease course.

Figure 5.2. The most frequent clinical manifestations at presentation (reported in ≥39% of patients) are shown above the grey dotted horizontal lines. [a]Number of patients ranged from 44 to 1137; number of studies ranged from 2-15; [b]Number of patients ranged from 51 to 4061; number of studies ranged from 2-31; [c]Pneumonia and lower respiratory tract infections were not combined into one category, as they were often mentioned as two separate categories in the included studies; [d]The prevalence of this clinical manifestation is similar or lower to lifetime prevalence estimates in general population.

Abbreviations: IBD, inflammatory bowel disease; inf, infections; ITP, idiopathic thrombocytopenic purpura; AIHA, autoimmune haemolytic anaemia; SLE, systemic lupus erythematosus.

Manifestation	Nr. of patients (studies)	Pooled proportion, % (95% CI)	I squared
Clinical phenotype[a]			
Infections only	1677 (16)	48 (39-58)	93
PLI	2252 (9)	29 (22-37)	92
Autoimmunity	4061 (28)	27 (22-32)	91
Malignancy[b]	3389 (20)	10 (7-14)	87
Enteropathy	2400 (10)	9 (6-13)	86
Lymphoid malignancy	3392 (20)	5 (3-7)	81
Respiratory involvement			
Respiratory tract infections	543 (5)	92 (78-99)	93
Upper RTI	794 (15)	84 (75-92)	89
Lower RTI	270 (8)	78 (64-90)	84
Sinusitis	1887 (21)	67 (57-77)	95
Pneumonia[c]	1876 (22)	62 (54-70)	92
Bronchitis	1166 (8)	62 (44-78)	97
Chronic lung disease	716 (3)	44 (27-63)	93
Otitis	1444 (18)	43 (35-51)	86
COPD	51 (2)	34 (14-58)	61
Allergy	419 (6)	33 (13-56)	94
Bronchiectasis	3720 (31)	32 (26-38)	93
Asthma	1819 (15)	25 (17-35)	94
Allergic rhinitis	1282 (7)	18 (8-31)	95
Pharyngitis/tonsillitis	575 (3)	18 (12-24)	68
GLILD	2094 (11)	15 (7-25)	96
ILD	629 (4)	13 (6-23)	88
Idiopathic lung fibrosis	247 (6)	12 (1-30)	92
Lymphoid hyperplasia	1667 (11)	10 (5-17)	91
Conjunctivitis	872 (6)	10 (5-16)	87
Gastrointestinal involvement			
Increased IEL	53 (1)	60	n/a
Gastrointestinal tract infection[d]	1566 (14)	29 (21-38)	92
Gastritis	286 (3)	28 (22-35)	17
Chronic diarrhoea	1885 (14)	27 (21-34)	88
Gastric metaplasia	53 (1)	25	n/a
Food intolerance	32 (1)	19	n/a
GERD[b]	565 (3)	16 (6-25)	84
Malabsorption	1099 (5)	13 (4-26)	96
Villous atrophy	291 (2)	11 (1-28)	83
Inflammatory bowel disease	1940 (14)	10 (6-16)	90
Helicobacter pylori	620 (4)	9 (3-18)	82
Lymphocytic colitis	92 (2)	9 (4-16)	0
Atrophic gastritis	652 (7)	5 (3-9)	62
Celiac disease	1586 (9)	3 (2-4)	25
Gastric cancer	1754 (11)	2 (1-4)	61
Hepatic involvement			
Unexplained hepatomegaly	1507 (8)	14 (8-22)	93
Urinary tract involvement			
Urinary tract infection[b]	1267 (12)	14 (11-18)	66
Pyelonephritis	205 (2)	8 (0-22)	77
Hematologic involvement			
Autoimmune cytopenia	3131 (17)	13 (10-17)	86
Unknown/other anaemia	988 (1)	10	n/a
Iron deficiency anaemia[b]	1322 (2)	10 (2-22)	97
ITP	2717 (18)	10 (7-14)	84
Bloodstream infection	1872 (16)	9 (5-13)	85
AIHA	2895 (20)	6 (4-8)	77
Autoimmune neutropenia	1944 (9)	4 (2-6)	68
Pernicious anaemia	2142 (10)	3 (1-5)	84
Neurologic involvement			
Headaches/migraine[b]	457 (1)	17	n/a
Infectious meningitis	1672 (17)	7 (5-9)	62

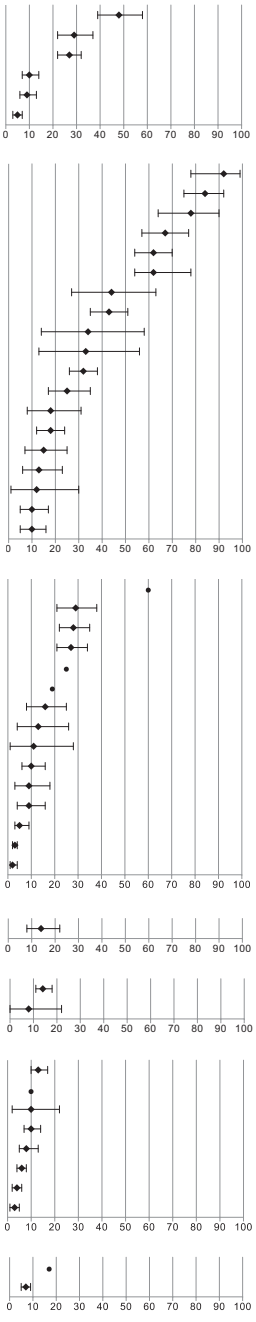


Figure 5.3. Frequency of reported clinical manifestations of patients with CVID.

Bone and joint involvement				
Arthralgia	457 (1)	20	n/a	
Osteoporosis/osteopenia[b]	480 (2)	10 (1-25)	92	
Septic arthritis	719 (7)	4 (1-8)	72	
Osteomyelitis	807 (6)	4 (1-8)	83	
Rheumatoid arthritis	1922 (8)	3 (2-4)	42	
Mastoiditis	532 (3)	3 (1-7)	64	
Lymph node and spleen involvement				
Lymphadenopathy	2122 (13)	30 (20-42)	96	
Splenomegaly	3153 (22)	29 (22-37)	94	
Non-malignant lymphoproliferation	1462 (6)	28 (16-40)	94	
Non-Hodgkin's lymphoma	2698 (13)	4 (3-5)	56	
Leukaemia[b]	1581 (4)	1 (1-2)	22	
Muco/cutaneous involvement				
Neutrophilic dermatitis	31 (1)	23	n/a	
Clubbing	138 (2)	19 (0-73)	97	
Aphthous lesions	147 (3)	16 (5-31)	77	
Skin infections	1033 (11)	13 (10-17)	59	
Atopic dermatitis	1307 (6)	12 (7-18)	86	
Cutaneous abscesses	72 (6)	5 (2-10)	79	
Vitiligo	2440 (12)	5 (3-8)	81	
Alopecia	1425 (6)	3 (2-4)	0	
Other clinical manifestations				
Fatigue	457 (1)	39	n/a	
Growth retardation/weight loss	759 (8)	13 (7-22)	87	
Unexplained granuloma[e]	2348 (11)	12 (9-16)	79	

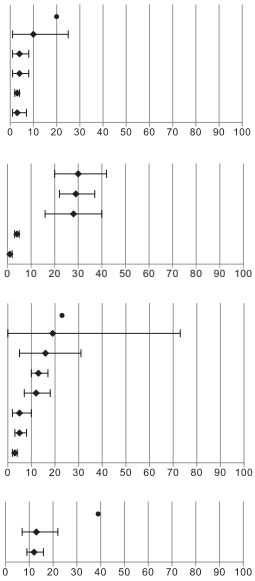


Figure 5.3. Continued.

Figure 5.3. All clinical manifestations that were present in ≥10% of patients are shown; the CVID-associated manifestations are also shown when present in <10% when they were considered important to incorporate by the authors (based on current literature and consensus in the field).

[a]Derived from Chapel et al. [26]; [b]The prevalence of this clinical manifestation is similar or lower to lifetime prevalence estimates in general population; [c]Streptococcus pneumoniae 15%, 95% CI 8-23; Hemophilus influenzae 19%, 95% CI 8-33; Moraxella catarrhalis 7%, 95% CI 0-19; Staphylococcus aureus 7%, 95% CI 3-12; Mycobacterial infection 1%, 95% CI 0-2; Pneumocystis jiroveci 1%, 95% CI 0-2%; Pseudomonas 6%, 95% CI 2-10; Aspergillus 3%, 95% CI 1-5; Mycoplasma 2%, 95% CI 0-4 ; [d]Giardia intestinalis 13%, 95% CI 7-21; Candida species 10%, 95% CI 4-19; Salmonella species 6%, 95% CI 2-12; Campylobacter species 4%, 95% CI 1-8 ; [e]Intestinal granulomatosis 1%, 95% CI 0-4; liver granuloma 3%, 95% CI 1-6; granuloma in lymph node 2%, 95% CI 0-5; granuloma in spleen 1%, 95% CI 0-2; skin granuloma 1%, 95% CI 0-2.

Abbreviations: AIHA, autoimmune haemolytic anaemia; COPD, chronic obstructive pulmonary disease; CVID, common variable immunodeficiency ; GERD, gastro-oesophageal reflux disease; GLILD, granulomatous and lymphocytic interstitial lung disease; IEL, increased intraepithelial lymphocytes; ILD, interstitial lung disease; ITP, idiopathic thrombocytopenic purpura; PLI, polyclonal lymphocytic infiltration; RTI, respiratory tract infection.

Figure 5.4. Frequency of reported clinical manifestations in children vs adults.

Figure 5.4. Abbreviations: IBD, inflammatory bowel disease; inf, infections; GLILD, granulomatous and lymphocytic interstitial lung disease; ITP, idiopathic thrombocytopenic purpura; AIHA, autoimmune haemolytic anaemia. ►

Manifestation	CHILDREN Pooled proportion, % (95% CI)	ADULTS Pooled proportion, % (95% CI)
Clinical phenotype		
Infections only	76 (57-91)	38 (27-49)
Autoimmunity	14 (5-27)	21 (12-30)
Enteropathy	10 (3-20)	14 (10-18)
Lymphoid malignancy	5 (2-10)	6 (5-8)
Malignancy	5 (3-7)	16 (11-21)
Respiratory involvement		
Otitis	55 (45-64)	32 (27-37)
Sinusitis	62 (50-74)	74 (60-86)
Upper respiratory tract inf	69 (45-89)	79 (66-90)
Pneumonia	61 (50-71)	66 (51-79)
Bronchitis	59 (11-71)	72 (38-97)
Bronchiectasis	16 (9-25)	36 (27-46)
GLILD	6 (4-9)	33 (13-57)
Lymphoid hyperplasia	2 (0-6)	11 (2-24)
Asthma	3 (5-9)	33 (21-47)
Interstitial lung disease	6 (1-14)	15 (3-35)
Gastrointestinal involvement		
Chronic diarrhoea	17 (11-24)	34 (22-48)
Gastrointestinal tract inf	35 (18-55)	34 (8-65)
IBD	3 (1-6)	18 (6-33)
Celiac disease	3 (1-5)	2 (1-4)
Malabsorption	5 (3-8)	11 (0-32)
Hepatic involvement		
Autoimmune hepatitis	2 (0-6)	4 (0-12)
Urinary tract involvement		
Urinary tract infection	11 (8-15)	15 (12-19)
Hematologic involvement		
ITP	9 (2-21)	6 (2-12)
Autoimmune cytopenia	15 (4-30)	16 (7-27)
AIHA	6 (4-9)	5 (2-8)
Autoimmune neutropenia	4 (0-11)	2 (0-6)
Pernicious anemia	1 (0-3)	1 (1-3)
Bloodstream infection	8 (5-12)	12 (9-16)
Neurologic involvement		
Infectious meningitis	7 (2-13)	7 (4-9)
Lymph node and spleen involvement		
Splenomegaly	20 (6-39)	30 (16-46)
Non-hodgkin's lymphoma	1 (0-3)	5 (4-6)
Hodgkin's lymphoma	5 (2-11)	1 (0-2)
Endocrinological involvement		
Thyroid disease	8 (0-24)	29 (12-50)
Autoimmune thyroiditis	3 (1-6)	6 (2-12)
Diabetes mellitus	2 (0-4)	4 (0-14)
Muco/cutaneous involvement		
Psoriasis	1 (0-6)	4 (2-7)
Vitiligo	2 (1-4)	2 (1-4)
Warts	6 (3-9)	5 (3-7)
Skin cancer	1 (0-3)	5 (2-8)
Skin infections	16 (9-25)	13 (6-23)
Recurrent HZ infections	19 (12-27)	7 (2-15)
Oral candidiasis	12 (8-16)	6 (0-20)
Other clinical manifestations		
Unexplained granuloma	6 (2-12)	15 (7-25)
Vasculitis	1 (0-5)	2 (2-4)

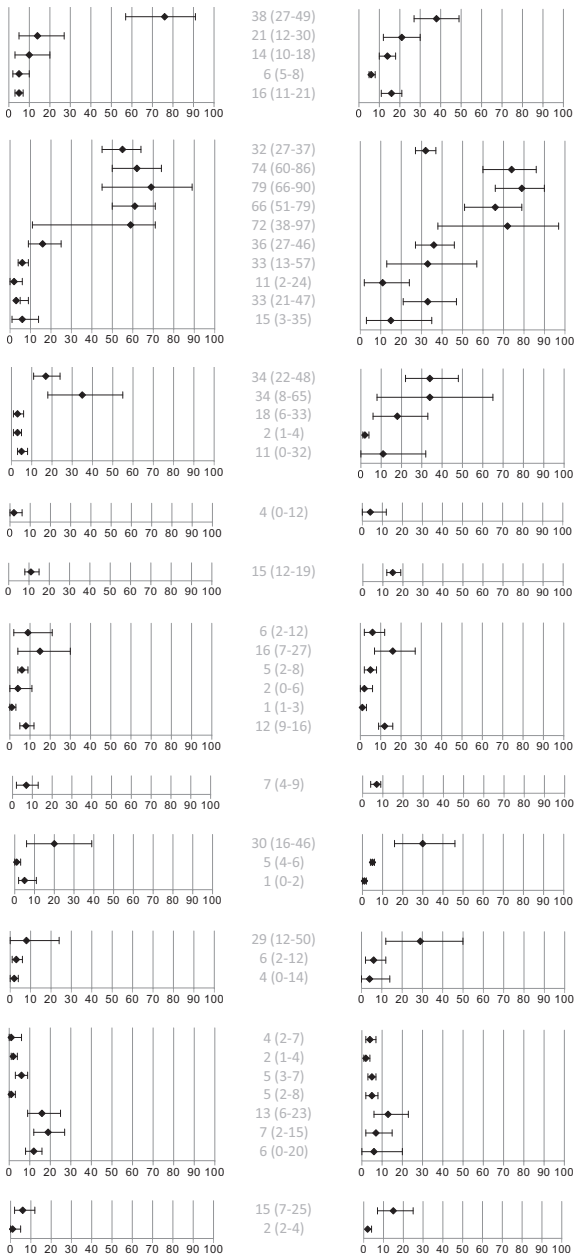


Table 5.2. Demographic parameters in adult-, paediatric-, and total cohorts.

	Children		
	Number of patients	Pooled proportion (95% CI)	I squared
Males	305/526	62 (54-69)	62
Females	221/526	38 (31-46)	62
Family members with PID	31/291	10 (6-15)	41
Consanguinity	60/118	31 (0-87)	96

Table 5.2. [a]Meta-analysis could not be conducted because the feature was described in only one study. Abbreviations: PID, primary immunodeficiency disease.

Three quarters of the children (76%, 95% CI 57-91) developed no other complications besides infections during the reported follow-up periods (See **Figure 5.4**), while this percentage was much lower in the adults (38%, 95% CI 27-49). Certain infectious features of CVID, such as otitis, were more common in children (55%, 95% CI 45-64) than in adults (32%, 95% CI 27-37), whereas certain immune dysregulation features, such as granulomatous-lymphocytic interstitial lung disease, chronic diarrhoea and inflammatory bowel disease were more prominent in adults (33%, 95% CI 13-57; 34%, 95% CI 22-48; 18%, 95% CI 6-33; respectively) than in children (6%, 95% CI 4-9; 17%, 95% CI 11-24; 3%, 95% CI 1-6, respectively). Bronchiectasis were more common in adults (36%, 95% CI 27-46) than in children (16%, 95% CI 9-25). An overview of all reported clinical manifestations in children and adults is included in **Supplementary Table 5.6**.

	Adults			Total cohort		
	Number of patients	Pooled proportion (95% CI)	I squared	Number of patients	Pooled proportion (95% CI)	I squared
	571/1326	43 (37-48)	71	1819/3828	50 (47-54)	71
	755/1326	58 (53-64)	72	2009/3828	50 (46-53)	73
	83/497	14 (9-20)	56	168/1283	12 (9-16)	66
	10	0[a]		238/769	20 (4-43)	97

DISCUSSION

To our knowledge, our study is the first systematic review and meta-analysis of pooled clinical manifestations in patients with CVID. Our findings can help clinicians to recognize CVID, and to estimate how common a clinical manifestation is in paediatric and adult CVID. We identified 134 different *presenting* clinical manifestations in patients diagnosed with CVID (the limited number of data impeded splitting up between children and adults). In addition, we identified 270 different clinical manifestations occurring during the entire course of the disease (147 in children and 170 in adults). Most frequent presenting manifestations were recurrent upper respiratory tract infections (73%), lower respiratory tract infections (73%), sinusitis (59%), pneumonia (57%), bronchitis (57%) and otitis (39%), concurrent with the first two ESID and first three JMF warning signs for PID [20,21]. However, these manifestations are also frequent in the general population and may lack discriminating value, unless their unusually recurrent and persistent nature is recognized [85]. Other alerts to potential PID that are in line with the JMF warning signs that may be more discriminatory include severe bacterial infections (osteomyelitis in 4%, meningitis in 6%, septicaemia in 8%, mastoiditis in 8%), which are clearly more frequent than lifetime prevalence in the general population. Recent studies already demonstrated a high incidence of antibody deficiency in patients with pneumococcal meningitis [86–88], confirming our finding of a high frequency of infectious meningitis in CVID, but we also show a high incidence of bloodstream infections, mastoiditis and to a somewhat lesser extent osteomyelitis in CVID patients. This suggests that the incidence of CVID may also be increased in patients with bloodstream infections, mastoiditis and osteomyelitis without other clear predispositions and suggest screening for CVID could be useful in these patients. This finding warrants further exploration.

One of the most reliable alerts to potential CVID was CVID in the family (12% of the total reviewed population). Both the six ESID and ten JMF warning signs make no mention of other presenting manifestations than frequent and/or severe infections, such as bronchiectasis (28%), lymphadenopathy (27%), splenomegaly (13%), chronic diarrhoea (21%), inflammatory bowel disease (11%), or autoimmune haematological manifestations (autoimmune cytopenia (10%) and idiopathic thrombocytopenia (6%)). Our results suggest that also lymphoproliferative, gastrointestinal and autoimmune manifestations should be included in warning signs for predicting PID. This is important, because we still fail to detect the disease early enough. Increasing awareness of this varied and complex presentation of CVID can lead to earlier detection and initiation of treatment.

Our findings show a male predominance in children with CVID (62%), but a female predominance in adults (58%). This is also observed in atopic disease and we previously described this in patients with unclassified primary antibody deficiency [89], but it has not previously been recognized in CVID. This sex shift may indicate that aetiology differs in different age groups. Early childhood male predominance suggests X-linked heredity is

present in some boys diagnosed with the disease; adult female predominance suggests sex hormone effects, environmental exposure, and epigenetic influences may play a role [90]. This implicates that future studies that attempt to define mechanisms that underpin CVID should be stratified according to sex.

There were clear differences in clinical manifestations occurring during the disease course between children and adults with CVID. Overall prevalence of bronchiectasis was 36% in adults vs 16% in children. Persistence of an 'infection-only' phenotype was much more prevalent in paediatric than in adult CVID (76 vs 38%). During childhood, three quarters of patients developed no other complications besides infections, while this percentage was much lower during follow up in adults (38%). Immune-dysregulation features, such as granulomatous-lymphocytic interstitial lung disease (15 vs 6%), chronic diarrhoea (34 vs 17%), and inflammatory bowel disease (18 vs 3%) were more prominent in adults compared to children. A possible explanation could be longer ongoing inflammation and longer follow-up in the adults [62]. One is an adult for many more years than one is a child, thus there are many more physician visits in the adult years and more opportunities for CVID complications to be observed. The different signs and symptoms observed in CVID between paediatric and adult age, with more non-infectious disease complications in adults, suggest that different monitoring strategies for children and adults during follow-up may be warranted.

Most common non-infectious manifestations included bronchiectasis (32%), lymphadenopathy (30%), splenomegaly (29%), polyclonal lymphocytic infiltration (29%), and autoimmune manifestations (27%). While only a quarter of CVID patients had features of immune dysregulation at presentation, this increased to about half of the patients throughout the course of the disease. This suggests that these manifestations more often occur later in the disease course. It is crucial that CVID patients are monitored for the development of these complications, because some of these are difficult to treat and associated with increased mortality (4,14). The coincidence of immunodeficiency and immune dysregulation can be explained by several mechanisms. Immunodeficiency may result in insufficient clearance of microbial antigens, and the resulting persistent antigenic exposure could then trigger granulomatous disease and autoimmunity [91]. Both complications have been linked to hyperplastic germinal centres enriched with polyclonal/self-reactive B-cell clones [92], and immature B cell development [25] in CVID. In addition, low numbers of regulatory T-cells [91,93], and an increasing number of genetic defects [94] have been associated with immune dysregulation in CVID. Additional factors, such as commensal microbial dysbiosis and epigenetic modifications remain to be better elucidated [95].

Interestingly, we found high pooled prevalence's of atopic diseases both at presentation of CVID and during the entire disease course (asthma 31 vs 25%, allergic rhinitis 36 vs 18%). The pooled prevalence's of asthma and allergic rhinitis are higher in CVID compared to the estimated lifetime prevalence's in the general population of 13.6% [96] and 6.6% [97], respectively. This should be interpreted with caution because of the considerable

heterogeneity between the studies (I^2 >80). Also, not all patients underwent cutaneous or in vitro testing or spirometry to support these diagnoses, nor was it reported how often the asthma was atopic in nature. It is possible that symptoms derived from the deficient immune system were interpreted as atopic disease, on the other hand, atopic disorders could actually be more prevalent in CVID. Overlap between the symptoms of atopic diseases and immunodeficiency may lead to delayed diagnosis, so it is important to consider CVID in patients with atopic diagnoses who are insufficiently responsive to standard treatment and who also have infections. To further elucidate the association between atopic diseases and CVID, a prospective multi-centre study in a large unselected CVID cohort would be needed.

A substantial number of patients developed malignancies during the disease course (10%, 95% CI 7-14). This pooled prevalence is comparable with the result of a previous focused meta-analysis of malignancy prevalence in CVID (8.6%, 95% CI 7.1-10) [98]. Also, in alignment with previous reports the most common malignancies were lymphoid malignancies (5%, 95% CI 3-7) and gastric cancers (2%, 95% CI 1-4) [71,72,98–100]. The lack of data on controls impedes comparison of our results to the normative population, but the prevalence's are higher compared to the lifetime prevalence estimates in the United States population (lymphoid malignancies 2.3%, gastric cancers 0.8%) [101]. The prevalence of cases with lung-, colorectal-, uterine-, liver-, and pancreatic cancer and leukaemia were similar to what one might expect in the general population according to the lifetime risk statistics based on the United States population (**Supplementary Table 5.4**) [101].

Strengths and limitations

This analysis collates data from >8000 patients (850 children, 2998 adults, 4673 not specified/both) in 51 studies from 18 different countries. The included studies were conducted in Europe, North- and South America, and Asia; there were no studies from Australia or Africa. Our review adhered to rigorous methods, including a systematic search strategy, and explicit inclusion criteria [102]. The findings therefore present the most comprehensive and internationally relevant presenting manifestations for clinicians worldwide.

The study has some limitations and potential sources of bias. The main limitations reflect deficits in the design and reporting of the included studies. Accuracy of our systematic analysis depends on the quality of the published and supplementary data that we included. All studies provided data on cases only, and not on controls. Therefore, we were unable to compare the frequency of clinical manifestations in CVID patients to the frequency in the general population. Publication bias could have led to overrepresentation of more complex cases of CVID, and therefore higher incidences of non-infectious complications. We did not include unpublished data. Heterogeneity between included studies was high. Most included studies provided little motivation for the selection of the clinical manifestations studied, thus it is difficult to account with certainty for the variation in number and choice of the selected clinical manifestations. The variation in reported clinical phenotypes and complications between cohorts may stem

from differences in study populations (for instance, due to access to health care, rate at which patients are properly diagnosed, degree of consanguinity, or population genetic differences), use of different methods to diagnose findings, underreporting of histological diagnoses because biopsies are not performed, and the use of different definitions for CVID. Full consensus regarding the definition of CVID does not yet exist [103]. Also, in a few series, a small proportion of paediatric and adult patients had opportunistic infections and/or a low CD4 T-cell count, and those patients should actually be classified as a combined immunodeficiency. This phenotype has been re-named in adults as late onset combined immunodeficiency (LOCID) by the IUIS. In addition, given the rapid progress in next-generation sequencing, in non-consanguineous populations, a causative mutation may currently be identified in ~25% of CVID patients [104]. Ideally, patients with monogenic diseases and LOCID should have been excluded from our analysis, but it was not possible to identify them exactly in the described cohorts [105]. Transient hypogammaglobulinemia of infancy may have been misdiagnosed as CVID in some of the children included in the different series. As we only included children diagnosed at age >4 years it is unlikely this accounts for a large percentage of included children.

Implications for future research

Our study identified two key limitations in the current evidence base on CVID presentation. First, we found relatively few studies that explicitly reported data on clinical signs and symptoms at or before diagnosis of the disease. We lack data on the frequency and time of onset of symptoms from the first symptoms at home to the final diagnosis. Second, only few studies compared paediatric with adult CVID. Further large, multicentre, prospective cohort studies, separately describing children and adults, would address these gaps.

Conclusions

In conclusion, this meta-analysis confirms the high frequency of upper and/or lower respiratory tract infections in CVID at presentation, but also shows a remarkably high incidence of severe bacterial infections (osteomyelitis in 4%, meningitis in 6%, septicaemia in 8%, mastoiditis in 8%) compared to lifetime prevalence in the general population. This suggests that the incidence of CVID may also be high in patients with severe bacterial infections without other clear predispositions and suggests screening for CVID might be useful in these patients. These findings warrant further exploration. In addition, CVID patients commonly present with other manifestations than frequent or severe infections – which are not included in ESID and JMF warning signs for identifying patients with primary immunodeficiencies. Not only the infectious, but also the immune dysregulation features (shown in **Figure 5.2**), should alert to the possibility of CVID, regardless whether they occur with or without recurrent infections. The bimodal sex distribution in patients with CVID implicates that future studies that attempt to define mechanisms that underpin CVID should be stratified according to sex.

REFERENCES

1. Picard C, Bobby Gaspar H, Al-Herz W, Bousfiha A, Casanova J-L, Chatila T, et al. International Union of Immunological Societies: 2017 Primary Immunodeficiency Diseases Committee Report on Inborn Errors of Immunity. J Clin Immunol. 2018 Jan;38(1):96–128.
2. Bonilla FA, Barlan I, Chapel H, Costa-Carvalho BT, Cunningham-Rundles C, de la Morena MT, et al. International Consensus Document (ICON): Common Variable Immunodeficiency Disorders. J allergy Clin Immunol Pract. 2016;4(1):38–59.
3. Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). Clin Immunol. 1999 Dec;93(3):190–7.
4. Odnoletkova I, Kindle G, Quinti I, Grimbacher B, Knerr V, Gathmann B, et al. The burden of common variable immunodeficiency disorders: a retrospective analysis of the European Society for Immunodeficiency (ESID) registry data. Orphanet J Rare Dis. 2018 Nov;13(1):201.
5. Resnick ES, Moshier EL, Godbold JH, Cunningham-Rundles C. Morbidity and mortality in common variable immune deficiency over 4 decades. Blood. 2012 Feb;119(7):1650–7.
6. Selenius JS, Martelius T, Pikkarainen S, Siitonen S, Mattila E, Pietikainen R, et al. Unexpectedly High Prevalence of Common Variable Immunodeficiency in Finland. Front Immunol. 2017;8:1190.
7. The French national registry of primary immunodeficiency diseases. Clin Immunol. 2010 May;135(2):264–72.
8. Matamoros Florí N, Mila Llambi J, Español Boren T, Raga Borja S, Fontan Casariego G. Primary immunodeficiency syndrome in Spain: first report of the National Registry in Children and Adults. J Clin Immunol. 1997 Jul;17(4):333–9.
9. Stray-Pedersen A, Abrahamsen TG, Frøland SS. Primary immunodeficiency diseases in Norway. J Clin Immunol. 2000 Nov;20(6):477–85.
10. Kilic SS, Ozel M, Hafizoglu D, Karaca NE, Aksu G, Kutukculer N. The prevalences [correction] and patient characteristics of primary immunodeficiency diseases in Turkey--two centers study. J Clin Immunol. 2013 Jan;33(1):74–83.
11. Edgar JDM, Buckland M, Guzman D, Conlon NP, Knerr V, Bangs C, et al. The United Kingdom Primary Immune Deficiency (UKPID) Registry: report of the first 4 years' activity 2008-2012. Clin Exp Immunol. 2014 Jan;175(1):68–78.
12. Marschall K, Hoernes M, Bitzenhofer-Grüber M, Jandus P, Duppenthaler A, Wuillemin WA, et al. The Swiss National Registry for Primary Immunodeficiencies: report on the first 6 years' activity from 2008 to 2014. Clin Exp Immunol. 2015 Oct;182(1):45–50.
13. Ludviksson BR, Sigurdardottir ST, Johannsson JH, Haraldsson A, Hardarson TO. Epidemiology of Primary Immunodeficiency in Iceland. J Clin Immunol. 2015 Jan;35(1):75–9.
14. Westh L, Mogensen TH, Dalgaard LS, Bernth Jensen JM, Katzenstein T, Hansen A-BE, et al. Identification and Characterization of a Nationwide Danish Adult Common Variable Immunodeficiency Cohort. Scand J Immunol. 2017 Jun;85(6):450–61.
15. Weifenbach N, Schneckenburger AAC, Lötters S. Global Distribution of Common Variable Immunodeficiency (CVID) in the Light of the UNDP Human Development Index (HDI): A Preliminary Perspective of a Rare Disease. J Immunol Res. 2020;2020:8416124.
16. Ilkjaer F V, Rasmussen LD, Martin-Iguacel R, Westh L, Katzenstein TL, Hansen A-BE, et al. How to Identify Common Variable Immunodeficiency Patients Earlier: General Practice Patterns. J Clin Immunol. 2019 Oct;39(7):641–52.

17. Quinti I, Di Pietro C, Martini H, Pesce AM, Lombardi F, Baumghartner M, et al. Health related quality of life in common variable immunodeficiency. Yonsei Med J. 2012 May;53(3):603–10.
18. Rider NL, Kutac C, Hajjar J, Scalchunes C, Seeborg FO, Boyle M, et al. Health-Related Quality of Life in Adult Patients with Common Variable Immunodeficiency Disorders and Impact of Treatment. J Clin Immunol. 2017 Jul;37(5):461–75.
19. Graziano V, Pecoraro A, Mormile I, Quaremba G, Genovese A, Buccelli C, et al. Delay in diagnosis affects the clinical outcome in a cohort of cvid patients with marked reduction of iga serum levels. Clin Immunol. 2017 Jul;180:1–4.
20. http://www.info4pi.org.
21. https://esid.org/Working-Parties/Clinical-Working-Party/Resources/6-Warning-Signs-for-PID-in-Adults [Internet]. [cited 2020 Apr 8]. Available from: https://esid.org/Working-Parties/Clinical-Working-Party/Resources/6-Warning-Signs-for-PID-in-Adults
22. Arkwright PD, Gennery AR. Ten warning signs of primary immunodeficiency: a new paradigm is needed for the 21st century. Ann N Y Acad Sci. 2011 Nov;1238:7–14.
23. Arslan S, Ucar R, Caliskaner AZ, Reisli I, Guner SN, Sayar EH, et al. How effective are the 6 European Society of Immunodeficiency warning signs for primary immunodeficiency disease? Ann Allergy Asthma Immunol. 2016 Feb;116(2):151-155.e1.
24. Gathmann B, Mahlaoui N, Gerard L, Oksenhendler E, Warnatz K, Schulze I, et al. Clinical picture and treatment of 2212 patients with common variable immunodeficiency. J Allergy Clin Immunol. 2014 Jul;134(1):116–26.
25. Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, et al. The EUROclass trial: defining subgroups in common variable immunodeficiency. Blood. 2008 Jan;111(1):77–85.
26. Chapel H, Lucas M, Lee M, Bjorkander J, Webster D, Grimbacher B, et al. Common variable immunodeficiency disorders: division into distinct clinical phenotypes. Blood. 2008 Jul;112(2):277–86.
27. Packwood K, Drewe E, Staples E, Webster D, Witte T, Litzman J, et al. NOD2 polymorphisms in clinical phenotypes of common variable immunodeficiency disorders. Clin Exp Immunol. 2010 Sep;161(3):536–41.
28. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA. 2000 Apr;283(15):2008–12.
29. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. BMJ. 2007 Oct;335(7624):806–8.
30. CASP. Casp Uk. 2017;(2018). Available from: www.casp-uk.net
31. Primary immunodeficiency diseases. Report of an IUIS Scientific Committee. International Union of Immunological Societies. Clin Exp Immunol. 1999 Oct;118 Suppl:1–28.
32. Primary immunodeficiency diseases. Report of a WHO scientific group. Clin Exp Immunol. 1997 Aug;109 Suppl:1–28.
33. Bonilla FA, Khan DA, Ballas ZK, Chinen J, Frank MM, Hsu JT, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. J Allergy Clin Immunol. 2015 Nov;136(5):1178–86.
34. Barendregt JJ, Doi S a. MetaXL User Guide. 2015;1–52.
35. Sanchez LA, Maggadottir SM, Pantell MS, Lugar P, Rundles CC, Sullivan KE. Two Sides of the Same Coin: Pediatric-Onset and Adult-Onset Common Variable Immune Deficiency. J Clin Immunol. 2017 Aug;37(6):592–602.

36. Baloh C, Reddy A, Henson M, Prince K, Buckley R, Lugar P. 30-Year Review of Pediatric- and Adult-Onset CVID: Clinical Correlates and Prognostic Indicators. J Clin Immunol. 2019 Oct;39(7):678–87.
37. Bezrodnik L, Gaillard MI, Carelli D. Clinical and immunological assessment of 94 patients with primary humoral immunode fi ciency : Common variable immunode fi ciency , selective IgA de fi ciency and polysaccharide antibody de fi ciency syndrome. 2011;6:159–66.
38. Aghamohammadi A, Abolhassani H, Latif A, Tabassomi F, Shokuhfar T, Torabi Sagvand B, et al. Long-term evaluation of a historical cohort of Iranian common variable immunodeficiency patients. Expert Rev Clin Immunol. 2014 Oct;10(10):1405–17.
39. Almejun MB, Sajaroff E, Galicchio M, Oleastro M, Bernasconi A, Zelazko M, et al. Immunological characteristics and two novel mutations in TACI in a cohort of 28 pediatric patients with common variable immunodeficiency. J Clin Immunol. 2012 Feb;32(1):89–97.
40. Ardeniz O, Basoglu OK, Gunsar F, Unsel M, Bayraktaroglu S, Mete N, et al. Clinical and immunological analysis of 23 adult patients with common variable immunodeficiency. J Investig Allergol Clin Immunol. 2010;20(3):222–36.
41. Ardeniz O, Cunningham-Rundles C. Granulomatous disease in common variable immunodeficiency. Clin Immunol. 2009 Nov;133(2):198–207.
42. Arshi S, Nabavi M, Bemanian MH, Shakeri R, Taghvaei B, Ghalebaghi B, et al. Phenotyping and follow up of forty-seven Iranian patients with common variable immunodeficiency. Allergol Immunopathol (Madr). 2016;44(3):226–31.
43. Erdem SB, Gulez N, Genel F, Karaman S, Nacaroglu HT. Characteristics of the patients followed with the diagnosis of common variable immunodeficiency and the complications. Cent J Immunol. 2019;44(2):119–26.
44. Barton JC, Bertoli LF, Barton JC. Comparisons of CVID and IgGSD: referring physicians, autoimmune conditions, pneumovax reactivity, immunoglobulin levels, blood lymphocyte subsets, and HLA-A and -B typing in 432 adult index patients. J Immunol Res. 2014;2014:542706.
45. Bates CA, Ellison MC, Lynch DA, Cool CD, Brown KK, Routes JM. Granulomatous-lymphocytic lung disease shortens survival in common variable immunodeficiency. J Allergy Clin Immunol. 2004 Aug;114(2):415–21.
46. Bjelac JA, Blanch MB, Fernandez J. Allergic disease in patients with common variable immunodeficiency at a tertiary care referral center. Vol. 120, Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. United States; 2018. p. 90–2.
47. Boileau J, Mouillot G, Gerard L, Carmagnat M, Rabian C, Oksenhendler E, et al. Autoimmunity in common variable immunodeficiency: correlation with lymphocyte phenotype in the French DEFI study. J Autoimmun. 2011 Feb;36(1):25–32.
48. Boursiquot J-N, Gerard L, Malphettes M, Fieschi C, Galicier L, Boutboul D, et al. Granulomatous disease in CVID: retrospective analysis of clinical characteristics and treatment efficacy in a cohort of 59 patients. J Clin Immunol. 2013 Jan;33(1):84–95.
49. Chan H-Y, Yang Y-H, Yu H-H, Chien Y-H, Chiang L-L, Chiang B-L. Clinical characteristics and outcomes of primary antibody deficiency: a 20-year follow-up study. J Formos Med Assoc. 2014 Jun;113(6):340–8.
50. Cunningham-Rundles C, Bodian C. Common variable immunodeficiency: clinical and immunological features of 248 patients. Clin Immunol. 1999 Jul;92(1):34–48.
51. Farmer JR, Ong M-S, Barmettler S, Yonker LM, Fuleihan R, Sullivan KE, et al. Common Variable Immunodeficiency Non-Infectious Disease Endotypes Redefined Using Unbiased Network Clustering in Large Electronic Datasets. Front Immunol. 2017;8:1740.

52. Feuille EJ, Anooshiravani N, Sullivan KE, Fuleihan RL, Cunningham-Rundles C. Autoimmune Cytopenias and Associated Conditions in CVID: a Report From the USIDNET Registry. J Clin Immunol. 2018 Jan;38(1):28–34.
53. Filion CA, Taylor-Black S, Maglione PJ, Radigan L, Cunningham-Rundles C. Differentiation of Common Variable Immunodeficiency From IgG Deficiency. J allergy Clin Immunol Pract. 2019 Apr;7(4):1277–84.
54. Fischer A, Provot J, Jais J-P, Alcais A, Mahlaoui N. Autoimmune and inflammatory manifestations occur frequently in patients with primary immunodeficiencies. J Allergy Clin Immunol. 2017 Nov;140(5):1388-1393.e8.
55. Van der Hilst JCH, Smits BW, van der Meer JWM. Hypogammaglobulinaemia: cumulative experience in 49 patients in a tertiary care institution. Neth J Med. 2002 Apr;60(3):140–7.
56. Jorgensen SF, Reims HM, Frydenlund D, Holm K, Paulsen V, Michelsen AE, et al. A Cross-Sectional Study of the Prevalence of Gastrointestinal Symptoms and Pathology in Patients With Common Variable Immunodeficiency. Am J Gastroenterol. 2016 Oct;111(10):1467–75.
57. Kainulainen L, Nikoskelainen J, Ruuskanen O. Diagnostic findings in 95 Finnish patients with common variable immunodeficiency. J Clin Immunol. 2001 Mar;21(2):145–9.
58. Karaulov A V, Sidorenko I V, Kapustina AS. Major approaches in early diagnostics of common variable immunodeficiency in adults in Moscow. F1000Research. 2012;1:46.
59. Khodadad A, Aghamohammadi A, Parvaneh N, Rezaei N, Mahjoob F, Bashashati M, et al. Gastrointestinal manifestations in patients with common variable immunodeficiency. Dig Dis Sci. 2007 Nov;52(11):2977–83.
60. Kokron CM, Errante PR, Barros MT, Baracho G V, Camargo MM, Kalil J, et al. Clinical and laboratory aspects of common variable immunodeficiency. An Acad Bras Cienc. 2004 Dec;76(4):707–26.
61. Llobet MP, Soler-Palacin P, Detkova D, Hernandez M, Caragol I, Espanol T. Common variable immunodeficiency: 20-yr experience at a single centre. Pediatr Allergy Immunol. 2009 Mar;20(2):113–8.
62. Maarschalk-Ellerbroek LJ, de Jong PA, van Montfrans JM, Lammers JWJ, Bloem AC, Hoepelman AIM, et al. CT screening for pulmonary pathology in common variable immunodeficiency disorders and the correlation with clinical and immunological parameters. J Clin Immunol. 2014 Aug;34(6):642–54.
63. Martinez Garcia MA, de Rojas MD, Nauffal Manzur MD, Munoz Pamplona MP, Compte Torrero L, Macian V, et al. Respiratory disorders in common variable immunodeficiency. Respir Med. 2001 Mar;95(3):191–5.
64. Mohammadinejad P, Aghamohammadi A, Abolhassani H, Sadaghiani MS, Abdollahzade S, Sadeghi B, et al. Pediatric patients with common variable immunodeficiency: long-term follow-up. J Investig Allergol Clin Immunol. 2012;22(3):208–14.
65. Mohammadinejad P, Pourhamdi S, Abolhassani H, Mirminachi B, Havaei A, Masoom SN, et al. Primary Antibody Deficiency in a Tertiary Referral Hospital: A 30-Year Experiment. J Investig Allergol Clin Immunol. 2015;25(6):416–25.
66. Musabak UH, Demirel F, Yesillik S, Baysan A, Selcuk A, Kartal O, et al. Adults with common variable immunodeficiency: a single-center experience. Turkish J Med Sci. 2017 Feb;47(1):1–12.
67. Ogershok PR, Hogan MB, Welch JE, Corder WT, Wilson NW. Spectrum of illness in pediatric common variable immunodeficiency. Ann Allergy Asthma Immunol. 2006 Nov;97(5):653–6.
68. Oksenhendler E, Gerard L, Fieschi C, Malphettes M, Mouillot G, Jaussaud R, et al. Infections in 252 patients with common variable immunodeficiency. Clin Infect Dis. 2008 May;46(10):1547–54.

69. Piatosa B, Pac M, Siewiera K, Pietrucha B, Klaudel-Dreszler M, Heropolitanska-Pliszka E, et al. Common variable immune deficiency in children--clinical characteristics varies depending on defect in peripheral B cell maturation. J Clin Immunol. 2013 May;33(4):731–41.
70. Piqueras B, Lavenu-Bombled C, Galicier L, Bergeron-van der Cruyssen F, Mouthon L, Chevret S, et al. Common variable immunodeficiency patient classification based on impaired B cell memory differentiation correlates with clinical aspects. J Clin Immunol. 2003 Sep;23(5):385–400.
71. Pulvirenti F, Pecoraro A, Cinetto F, Milito C, Valente M, Santangeli E, et al. Gastric Cancer Is the Leading Cause of Death in Italian Adult Patients With Common Variable Immunodeficiency. Front Immunol. 2018;9:2546.
72. Quinti I, Soresina A, Spadaro G, Martino S, Donnanno S, Agostini C, et al. Long-term follow-up and outcome of a large cohort of patients with common variable immunodeficiency. J Clin Immunol. 2007 May;27(3):308–16.
73. Ramirez-Vargas N, Arablin-Oropeza SE, Mojica-Martinez D, Yamazaki-Nakashimada MA, de la Luz Garcia-Cruz M, Teran-Juarez LM, et al. Clinical and immunological features of common variable immunodeficiency in Mexican patients. Allergol Immunopathol (Madr). 2014;42(3):235–40.
74. Santaella ML, Font I, Disdier O. Common variable immunodeficiency: experience in Puerto Rico. P R Health Sci J. 2005 Mar;24(1):7–10.
75. Sperlich JM, Grimbacher B, Workman S, Haque T, Seneviratne SL, Burns SO, et al. Respiratory Infections and Antibiotic Usage in Common Variable Immunodeficiency. J allergy Clin Immunol Pract. 2018 Jan;6(1):159-168.e3.
76. Thickett KM, Kumararatne DS, Banerjee AK, Dudley R, Stableforth DE. Common variable immune deficiency: respiratory manifestations, pulmonary function and high-resolution CT scan findings. QJM. 2002 Oct;95(10):655–62.
77. Urschel S, Kayikci L, Wintergerst U, Notheis G, Jansson A, Belohradsky BH. Common variable immunodeficiency disorders in children: delayed diagnosis despite typical clinical presentation. J Pediatr. 2009 Jun;154(6):888–94.
78. van de Ven AAJM, van de Corput L, van Tilburg CM, Tesselaar K, van Gent R, Sanders EAM, et al. Lymphocyte characteristics in children with common variable immunodeficiency. Clin Immunol. 2010 Apr;135(1):63–71.
79. Wiesik-Szewczyk E, Zietkiewicz M, Matyja-Bednarczyk A, Napiorkowska-Baran K, Suchanek H, Jahnz-Rozyk K. The first Polish cohort of adult patients with common variable immunodeficiency from 4 specialized centers: do we provide standards of care? Polish Arch Intern Med. 2018 Sep;128(9):563–6.
80. Yazdani R, Heydari A, Azizi G, Abolhassani H, Aghamohammadi A. Asthma and Allergic Diseases in a Selected Group of Patients With Common Variable Immunodeficiency. J Investig Allergol Clin Immunol. 2016;26(3):209–11.
81. Yong PL, Orange JS, Sullivan KE. Pediatric common variable immunodeficiency: immunologic and phenotypic associations with switched memory B cells. Pediatr Allergy Immunol. 2010 Aug;21(5):852–8.
82. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Dis Mon. 2003 Feb;49(2):53–70.
83. https://www.thyroid.org/media-main/press-room/.
84. Eaton WW, Pedersen MG, Atladóttir HO, Gregory PE, Rose NR, Mortensen PB. The prevalence of 30 ICD-10 autoimmune diseases in Denmark. Immunol Res. 2010 Jul;47(1–3):228–31.
85. de Vries E. Patient-centred screening for primary immunodeficiency, a multi-stage diagnostic protocol designed for non-immunologists: 2011 update. Clin Exp Immunol. 2012 Jan;167(1):108–19.

86. Butters C, Phuong LK, Cole T, Gwee A. Prevalence of Immunodeficiency in Children With Invasive Pneumococcal Disease in the Pneumococcal Vaccine Era: A Systematic Review. JAMA Pediatr. 2019 Sep;
87. Cowan J, Do TL, Desjardins S, Ramotar K, Corrales-Medina V, Cameron DW. Prevalence of Hypogammaglobulinemia in Adult Invasive Pneumococcal Disease. Clin Infect Dis. 2018 Feb;66(4):564–9.
88. Gaschignard J, Levy C, Chrabieh M, Boisson B, Bost-Bru C, Dauger S, et al. Invasive pneumococcal disease in children can reveal a primary immunodeficiency. Clin Infect Dis. 2014 Jul;59(2):244–51.
89. Janssen LMA, Bassett P, Macken T, van Esch J, Pruijt H, Knoops A, et al. Mild Hypogammaglobulinemia Can Be a Serious Condition. Front Immunol. 2018;9:2384.
90. Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. Front Neuroendocrinol. 2014 Aug;35(3):347–69.
91. Horn J, Manguiat A, Berglund LJ, Knerr V, Tahami F, Grimbacher B, et al. Decrease in phenotypic regulatory T cells in subsets of patients with common variable immunodeficiency. Clin Exp Immunol. 2009 Jun;156(3):446–54.
92. Romberg N, Le Coz C, Glauzy S, Schickel J-N, Trofa M, Nolan BE, et al. Patients with common variable immunodeficiency with autoimmune cytopenias exhibit hyperplastic yet inefficient germinal center responses. J Allergy Clin Immunol. 2019 Jan;143(1):258–65.
93. Fevang B, Yndestad A, Sandberg WJ, Holm AM, Muller F, Aukrust P, et al. Low numbers of regulatory T cells in common variable immunodeficiency: association with chronic inflammation in vivo. Clin Exp Immunol. 2007 Mar;147(3):521–5.
94. Ameratunga R, Lehnert K, Woon S-T, Gillis D, Bryant VL, Slade CA, et al. Review: Diagnosing Common Variable Immunodeficiency Disorder in the Era of Genome Sequencing. Clin Rev Allergy Immunol. 2018 Apr;54(2):261–8.
95. Jorgensen SF, Fevang B, Aukrust P. Autoimmunity and Inflammation in CVID: a Possible Crosstalk between Immune Activation, Gut Microbiota, and Epigenetic Modifications. J Clin Immunol. 2019 Jan;39(1):30–6.
96. https://ftp.cdc.gov/pub/Health_Statistics/NCHS/NHIS/SHS/2018_SHS_Table_A-2.pdf.
97. Ghouri N, Hippisley-Cox J, Newton J, Sheikh A. Trends in the epidemiology and prescribing of medication for allergic rhinitis in England. J R Soc Med. 2008 Sep;101(9):466–72.
98. Kiaee F, Azizi G, Rafiemanesh H, Zainaldain H, Sadaat Rizvi F, Alizadeh M, et al. Malignancy in common variable immunodeficiency: a systematic review and meta-analysis. Expert Rev Clin Immunol. 2019 Oct;15(10):1105–13.
99. Vajdic CM, Mao L, van Leeuwen MT, Kirkpatrick P, Grulich AE, Riminton S. Are antibody deficiency disorders associated with a narrower range of cancers than other forms of immunodeficiency? Blood. 2010 Aug;116(8):1228–34.
100. Kralickova P, Milota T, Litzman J, Malkusova I, Jilek D, Petanova J, et al. CVID-Associated Tumors: Czech Nationwide Study Focused on Epidemiology, Immunology, and Genetic Background in a Cohort of Patients With CVID. Front Immunol. 2018;9:3135.
101. https://seer.cancer.gov/statfacts/html/all.html.
102. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015 Jan;4:1.
103. Ameratunga R, Woon S-T. Perspective: Evolving Concepts in the Diagnosis and Understanding of Common Variable Immunodeficiency Disorders (CVID). Clin Rev Allergy Immunol. 2019 Nov;
104. Maffucci P, Filion CA, Boisson B, Itan Y, Shang L, Casanova J-L, et al. Genetic Diagnosis Using Whole Exome Sequencing in Common Variable Immunodeficiency. Front Immunol. 2016;7:220.

105. Malphettes M, Gerard L, Carmagnat M, Mouillot G, Vince N, Boutboul D, et al. Late-onset combined immune deficiency: a subset of common variable immunodeficiency with severe T cell defect. Clin Infect Dis. 2009 Nov;49(9):1329–38.

eSEARCH: ELECTRONIC DATABASE SEARCH STRATEGY

A. Embase search strategy for common variable immunodeficiency disease (last conducted January 2019)

((((‘common variable immunodeficiency’:ti,ab,kw OR ‘cvid’:ti,ab,kw OR ‘late onset hypogammaglobulinemia’:ti,ab,kw OR ‘late onset hypogammaglobulinaemia’:ti,ab,kw) AND [english]/lim AND [1999-2018]/py) NOT (‘animal cell’/de OR ‘animal experiment’/de OR ‘animal model’/de OR ‘case report’/de OR ‘human cell’/de OR ‘human tissue’/de OR ‘in vitro study’/de OR ‘model’/de OR ‘nonhuman’/de OR ‘a case of’:ti)) NOT (‘human immunodeficiency virus infection’ OR ‘acquired immune deficiency syndrome’ OR hiv)) NOT (‘in vitro’:ti,ab,kw OR ‘human cell’:ti,ab,kw OR ‘human tissue’:ti,ab,kw OR ‘mutation’:ti,ab,kw OR ‘cellular’:ti,ab,kw)

B. Cochrane search strategy for common variable immunodeficiency disease (last conducted January 2019)

(CVID OR “common variable immunodeficiency”) NOT (HIV OR AIDS OR “human immunodeficiency virus” OR “acquired immune deficiency syndrome”)

C. PubMed search strategy for common variable immunodeficiency disease (last conducted January 2019)

((((((((“Sign”[Journal] OR “sign”[All Fields]) OR (“diagnosis”[Subheading] OR “diagnosis”[All Fields] OR “signs”[All Fields] OR “diagnosis”[MeSH Terms] OR “signs”[All Fields])) OR (“diagnosis”[Subheading] OR “diagnosis”[All Fields] OR “symptoms”[All Fields] OR “diagnosis”[MeSH Terms] OR “symptoms”[All Fields])) OR clinical[All Fields]) OR (“protein domains”[MeSH Terms] OR (“protein”[All Fields] AND “domains”[All Fields]) OR “protein domains”[All Fields] OR “feature”[All Fields])) OR present[All Fields]) OR characteristic[All Fields]) OR manifestation[All Fields]) OR (clinical[All Fields] AND (“protein domains”[MeSH Terms] OR (“protein”[All Fields] AND “domains”[All Fields]) OR “protein domains”[All Fields] OR “feature”[All Fields]))) OR symptom[All Fields]) AND (((((((“common variable immunodeficiency”[Title/Abstract] OR CVID[Title/Abstract]) OR “common variable immunodeficiency disorders”[Title/Abstract]) OR “late onset hypogammaglobulinaemia”[Title/Abstract]) OR “late onset hypogammaglobulinemia”[Title/Abstract]) NOT ((((“hiv”[MeSH Terms] OR “hiv”[All Fields]) OR “human immunodeficiency virus”[All Fields]) OR “acquired immunodeficiency syndrome”[All Fields]) OR (“acquired immunodeficiency syndrome”[MeSH Terms] OR (“acquired”[All Fields] AND “immunodeficiency”[All Fields] AND “syndrome”[All Fields]) OR “acquired immunodeficiency syndrome”[All Fields] OR “aids”[All Fields])))) NOT ((“case reports”[Publication Type] OR “case report”[All Fields]) OR “case reports”[Publication Type])) AND (“1999/01/01”[PDAT] : “2018/12/31”[PDAT]) AND “humans”[MeSH Terms] AND English[lang]) AND (“1999/01/01”[PDAT] : “2018/12/31”[PDAT]) AND “humans”[MeSH Terms] AND English[lang]) AND ((“1999/01/01”[PDAT] : “2018/12/31”[PDAT]) AND “humans”[MeSH Terms] AND English[lang])

Supplementary Table 5.1. Handling overlapping data.

Problem	How this was dealt with
Multiple updates of a cohort were published with a complete overlapping recruitment period.	The study with the largest dataset describing the overall clinical picture of the cohort was included.
A cohort, originating from a single centre, that been extensively described and published, was later included in a registry or multicentre cohort report.	When there were overlapping variables between the single-centre and registry/multicentre-study, the overlapping variables were included only from the larger (and often more recent) multicentre study.
The same centre/ registry published an article about their total cohort and another article in which children and/or adults were separately described.	Children- and adult-specific overlapping data were only included in the subgroup analysis for children vs adults.
The study focused only on certain clinical manifestations.	-When this was the most recent update of the cohort, only the overlapping clinical manifestations were removed from earlier publications on the cohort(s). -When these studies with a focus were followed by studies reporting a larger cohort, which described the same clinical manifestations, this focused study was excluded.
A large multicentre cohort included cohorts from centres that already were previously published as single-centre cohort in more detail.	When only few clinical manifestations were described in the multicentre cohort, this large cohort was not included in the analysis and preference was given to the smaller cohorts which were described in more detail.
Multicentre studies with partial overlapping included centres.	The largest multicentre study describing the overall clinical picture of the cohort was included.

	Excluded studies	Included study	Studies of whom overlapping variables were excluded	Study of whom all variables were included
	Aghamohammadi 2006 Aghamohammadi 2010 Mokhtari 2016 Valizadeh 2017	Aghamohammadi 2014		
			Piqueras 2003	Boursiquot 2013
			Mohammadinejad 2015	Aghamohammadi 2014
			Graziano 2017	Pulvirenti 2018
			Cunningham-Rundles 1999 Ardeniz 2009 Resnick 2012 Filion 2018 Baloh 2019	Feuille 2018
			Mohammadinejad 2012	Aghamohammadi 2014
			Sanchez 2017 Farmer 2018	Feuille 2018
	Wang 2005 Zhang 2007	Resnick 2012	Oksenhendler 2007 Boileau 2011	Boursiquot 2013
			Khodadad 2007	Yazdani 2016 Aghamohammadi 2014
	Gathmann 2014			
	Wehr 2008 Packwood 2010		Chapel 2008	

Supplementary Table 5.3. Definitions of common variable immunodeficiency.

ESID/PAGID	ESID diagnostic criteria
Probable Marked decrease (≥2 SD below mean for age) in serum IgG and IgA and: 1.Onset of immunodeficiency ≥2 years of age 2.Absent isohemagglutinins and/or poor response to vaccines 3.Defined causes of hypogammaglobulinemia have been excluded	At least one of the following: -increased susceptibility to infection -autoimmune manifestations -granulomatous disease -unexplained polyclonal lymphoproliferation -affected family member with antibody deficiency AND marked decrease of IgG and IgA with or without low IgM levels;
Possible Marked decrease (≥2 SD below mean for age) in one of the major isotypes (IgM, IgG, and IgA) and: 1.Onset of immunodeficiency ≥2 years of age 2.Absent isohemagglutinins and/or poor response to vaccines 3.Defined causes of hypogammaglobulinemia have been excluded	AND at least one of the following: -poor antibody response to vaccines -low switched memory B cells (<70% of age-related normal value) AND secondary causes have been excluded (e.g., infection, protein loss, medication, malignancy)

Supplementary Table 5.3. Abbreviations: ESID, European Society for Immunodeficiencies; ICON, International Consensus Document; IUIS, International Union of Immunological Societies; PAGID, Pan-American Group for Immunodeficiency; WHO, World Health Organization.

	IUIS criteria	WHO scientific group	ICON
	Decreased serum levels of IgG, IgA, and/or IgM (≥2 SD below mean for age)	Decreased serum levels of IgG, IgA, and/or IgM (≥2 SD below mean for age)	Decreased serum levels of IgG, IgA, and/or IgM (≥2 SD below mean for age), impairment of specific antibody responses, and, occasionally, reductions in B-cell numbers

Supplementary Table 5.4. Subgroup analyses of clinical manifestations at presentation vs overall clinical manifestations during the disease course compared to population lifetime prevalence.

	At or before diagnosis	
Manifestation	**Pooled proportion (95% CI)**	**I^2**
Clinical phenotype[a]		
Infections only	93 (78-100)	74
Autoimmunity	19 (11-28)	81
Polyclonal lymphocytic infiltration	n/a	n/a
Enteropathy	n/a	n/a
Lymphoid malignancy	2 (0-4)	0
Malignancy	4 (1-8)	0
Respiratory involvement		
Otitis	39 (28-49)	88
Sinusitis	59 (47-71)	93
Upper respiratory tract infections	73 (53-89)	96
Pneumonia	57 (43-69)[c]	94
Bronchitis	57 (42-72)	92
Lower respiratory tract infections	73 (56-86)	85
Bronchiectasis	28 (18-40)	91
Lymphocytic interstitial pneumonia	n/a	n/a
GLILD	n/a	n/a
Lymphoid hyperplasia[e]	n/a	n/a
Asthma	31 (3-69)	92
Allergic rhinitis	36 (9-68)	82
Idiopathic lung fibrosis	n/a	n/a
Emphysema	4 (1-10)	0
Pulmonary lobe resection	5 (3-8)	0
Respiratory tract infections	86 (62-99)	94
Lung cancer	n/a	n/a
Allergy	16 (0-63)	96
Mastoiditis	8 (3-16)	0
Interstitial lung disease	n/a	n/a
Lung nodules	n/a	n/a
Pharyngitis/tonsillitis	n/a	n/a
Conjunctivitis	14 (6-25)	73
COPD	n/a	n/a

Overall		General population prevalence
Pooled proportion (95% CI)	**I^2**	**Lifetime prevalence percentage estimates from various sources**
48 (39-58)	93	n/a
27 (22-32)	91	3.9% (1)
29 (22-37)	92	< <1%
9 (6-13)	86	< <1%
5 (3-7)	81	2.1 + 0.2 = 2.3% (2)
10 (7-14)	87	39.5 (2)
43 (35-51)	86	n/a[b]
67 (57-77)	95	11.2% (3)
84 (75-92)	89	n/a[b]
62 (54-70)[d]	92	n/a[b]
62 (44-78)	97	n/a[b]
78 (64-90)	84	n/a
32 (26-38)	93	0.27% (4)
3 (1-5)	40	n/a
15 (7-25)	96	n/a
10 (5-17)	91	n/a
25 (17-35)	94	13.6% (3)
18 (8-31)	95	6.6% (5)
12 (1-30)	92	0.01% (6)
4 (1-10)	0	1.3% (3)
5 (3-8)	0	n/a
92 (78-99)	93	n/a
1 (0-1)	44	6.3% (2)
33 (13-56)	94	4.6 (7)
3 (1-7)	64	<0.1% (1.2/10,000 child years) (8)
13 (6-23)	88	n/a
3 (1-6)	53	n/a
18 (12-24)	68	n/a[b]
10 (5-16)	87	n/a[b]
34 (14-58)	61	1.7% (9)

Supplementary Table 5.4. Continued.

	At or before diagnosis	
Manifestation	**Pooled proportion (95% CI)**	**I^2**
Gastrointestinal involvement		
Chronic diarrhoea of unknown origin	21 (11-34)	92
Gastrointestinal tract infection	29 (15-44)[f]	72
Intestinal granulomatosis	n/a	n/a
Inflammatory bowel disease	11 (3-23)	59
Crohn's disease	n/a	n/a
Ulcerative colitis	n/a	n/a
Lymphocytic colitis	n/a	n/a
Eosinophilic inflammation	n/a	n/a
Celiac disease	1 (0-4)	23
Villous atrophy[h]	n/a	n/a
Atrophic gastritis	n/a	n/a
Protein losing enteropathy	n/a	n/a
Stomach cancer	n/a	n/a
Colorectal cancer	n/a	n/a
Gastro-oesophageal reflux disease	n/a	n/a
Gastritis	n/a	n/a
Peritonitis	3 (1-8)	0
Malabsorption	n/a	n/a
Glandular tissue		
Sjogren's syndrome	13 (2-25)	67
Breast cancer	n/a	n/a
Cervix cancer	n/a	n/a
Uterine cancer	n/a	n/a
Hepatic involvement		
Autoimmune hepatitis	n/a	n/a
Primary biliary cirrhosis	n/a	n/a
Unexplained hepatomegaly	n/a	n/a
Viral hepatitis	n/a	n/a
Liver granuloma	n/a	n/a
Liver cirrhosis	n/a	n/a
Liver cancer	n/a	n/a
Hepatitis (not further specified)	n/a	n/a

Overall		General population prevalence
Pooled proportion (95% CI)	**I²**	**Lifetime prevalence percentage estimates from various sources**
27 (21-34)	88	4-5% (10)
29 (21-38)[g]	92	n/a[b]
1 (0-4)	81	< <0.01%
10 (6-16)	90	0.71% (1)
3 (2-4)	0	0.23% (1)
2 (1-3)	0	0.48% (1)
9 (4-16)	0	n/a
5 (2-10)	0	< <1% (11)
3 (2-4)	25	0.06% (1)
11 (1-28)	83	n/a
5 (3-9)	62	n/a (12)
1 (0-3)	0	< <1% (13)
2 (1-4)	61	0.8% (2)
1 (0-2)	40	4.2% (2)
16 (8-25)	84	22.2% (14)
28 (22-35)	17	n/a
4 (1-8)	0	< <1%
13 (4-26)	96	n/a
3 (1-6)	86	0.06% (1)
1 (1-2)	39	12.9% (2)
1 (1-2)	0	0.6% (2)
1 (0-2)	28	3.1% (2)
4 (0-10)	89	0.045% (1)
2 (1-3)	39	< <1%
14 (8-72)	93	< <1%
4 (2-6)	64	n/a
3 (1-6)	68	n/a
4 (1-9)	0	n/a
1 (0-1)	44	1% (2)
4 (0-12)	94	n/a

Supplementary Table 5.4. Continued.

	At or before diagnosis	
Manifestation	**Pooled proportion (95% CI)**	**I^2**
Urinary tract involvement		
Urinary tract infection	14 (5-25)	83
Pyelonephritis	n/a	n/a
Hematologic involvement		
Idiopathic thrombocytopenia	6 (4-9)	20
Autoimmune cytopenia	10 (7-14)	0
Autoimmune haemolytic anaemia	3 (2-5)	0
Autoimmune neutropenia	3 (0-9)	48
Unknown/other neutropenia	n/a	n/a
Evans syndrome	n/a	n/a
Iron deficiency anaemia	n/a	n/a
Pernicious anaemia	n/a	n/a
Bloodstream infection	8 (4-13)	77
Neurological involvement		
Headache/migraine	n/a	n/a
Infectious meningitis	5 (3-9)[j]	76
Myasthenia gravis	n/a	n/a
Multiple sclerosis	n/a	n/a
Bell's palsy	n/a	n/a
Polyneuropathy	n/a	n/a
Epilepsy	n/a	n/a
Bone and joint involvement		
Septic arthritis	2 (1-4)	0
Rheumatoid arthritis	4 (1-10)	70
Seronegative arthritis	6 (2-12)	21
Juvenile idiopathic arthritis	n/a	n/a
Psoriatic arthritis	n/a	n/a
Osteomyelitis	4 (1-9)	16
Multiple myeloma	n/a	n/a
Kaposi's sarcoma	n/a	n/a
Osteoporosis/osteopenia	n/a	n/a

Overall		General population prevalence
Pooled proportion (95% CI)	**I^2**	**Lifetime prevalence percentage estimates from various sources**
14 (11-18)	66	30% (female 50%, male 12%) (15)
8 (0-22)	77	2% (15)
10 (7-14)	84	0.045% (1)
13 (10-17)	86	n/a
6 (4-8)	77	0.013% (1)
4 (2-6)	68	< < 0.1% (16)
6 (3-10)	86	< < 0.1% (16)
2 (1-2)	0	< < 0.1% (17)
10 (2-22)	97	12.2% (18)[i]
3 (1-5)	84	0.05% (1)
9 (5-13)	85	1.2% (19)
17 (n/a)	n/a	15.9% (20)
7 (5-9)[k]	62	0.3% (21)
0 (0-1)	47	0.018% (1)
0 (0-1)	22	0.19% (1)
1 (0-2)	52	0.6% (22)
3 (1-6)	64	1% (23)
6 (2-11)	71	10% (24)
4 (1-8)	72	0.15% (25)
3 (2-4)	42	0.5% (1)
4 (2-7)	74	21.4% (26)
1 (0-4)	82	0.073% (1)
0 (0-1)	47	n/a
4 (1-8)	83	1.7% (27)
0 (0-1)	0	n/a
0 (0-1)	0	n/a
10 (1-25)	92	27% (28)

Supplementary Table 5.4. Continued.

	At or before diagnosis	
Manifestation	**Pooled proportion (95% CI)**	**I^2**
Lymph node and spleen involvement		
Lymphadenopathy	27 (0-90)	96
Non-malignant lymphoproliferation	n/a	n/a
Splenomegaly	13 (5-23)	90
Non-Hodgkin's lymphoma	n/a	n/a
Splenectomy	3 (1-6)	0
Pancreatic cancer	n/a	n/a
Leukaemia	n/a	n/a
Granuloma in lymph node	n/a	n/a
Granuloma in spleen	n/a	n/a
Granuloma in bone marrow	n/a	n/a
Endocrinological involvement		
Thyroid disease (all)	16 (0-43)	92
Autoimmune thyroiditis	3 (0-8)	68
Diabetes mellitus	2 (0-7)	0
Thyroid cancer	n/a	n/a
Muco/cutaneous involvement		
Psoriasis	n/a	n/a
Atopic dermatitis	12 (4-24)	0
Alopecia	n/a	n/a
Vitiligo	2 (1-5)	0
Warts	n/a	n/a
Skin cancer	n/a	n/a
Skin infections	10 (6-15)	43
Cutaneous abscesses	3 (1-6)	17
(Recurrent) herpes zoster	n/a	n/a
(Recurrent) herpes simplex	n/a	n/a
Oral candidiasis	3 (1-5)	0
Genital candidiasis	n/a	n/a
Urticaria	n/a	n/a
Oral/dental infections	10 (3-21)	0
Skin granuloma	n/a	n/a
Severe varicella	n/a	n/a
Recurrent parotitis	n/a	n/a
Clubbing	n/a	n/a
Aphthous lesions	n/a	n/a

Overall		General population prevalence
Pooled proportion (95% CI)	**I^2**	**Lifetime prevalence percentage estimates from various sources**
30 (20-42)	96	64% (29)
28 (16-40)	94	n/a
29 (22-37)	94	2% (30)
4 (3-5)	56	2.1% (2)
6 (3-8)	57	n/a
2 (0-7)	57	1.6% (2)
1 (1-2)	22	1.5% (2)
2 (0-5)	80	n/a
1 (0-2)	24	n/a
0 (0-1)	0	n/a
9 (4-16)	95	12% (31)
4 (2-6)	80	0.058% (1)
2 (1-3)	18	0.9% (type 1) (1)
1 (0-3)	68	1.3% (2)
3 (2-5)	38	0.32% (1)
12 (7-18)	86	2.1-4.9% (32)
3 (2-4)	0	0.031% (1)
5 (3-8)	81	0.022% (1)
5 (4-7)	0	n/a
3 (1-5)	70	20% (33)
13 (10-17)	59	n/a
5 (2-10)	79	n/a
9 (5-14)	84	23.8% (34)
9 (2-10)	89	38.3% (35)
6 (2-11)	91	n/a
6 (3-12)	0	30-50% (36)
5 (3-8)	71	11.5% (37)
4 (3-6)	5	n/a
1 (0-2)	41	n/a
4 (2-7)	0	n/a
6 (0-3)	93	n/a
19 (0-73)	97	n/a
16 (5-31)	77	0.1-1.2% (38)

Supplementary Table 5.4. Continued.

	At or before diagnosis	
Manifestation	**Pooled proportion (95% CI)**	**I^2**
Other clinical manifestations		
Growth retardation/weight loss	8 (1-22)	82
Unexplained granuloma	5 (4-7)	0
Vasculitis	n/a	n/a
Systemic lupus erythematosus	2 (0-6)	28
Asymptomatic	6 (2-11)	74

Supplementary Table 5.4. [a]According to Chapel et al. (39); [b]The lifetime prevalence of respiratory and gastrointestinal tract infections is also high in the general population and may lack any discriminating value, unless their unusually recurrent and persistent nature is recognized. Because of the absence of data on the number of infectious episodes in the included studies, population lifetime prevalence percentages are not shown. [c]Streptococcus pneumoniae 11% (6-19), Hemophilus influenzae 17% (0-49), mycobacterial infection 5% (2-8); [d]Streptococcus pneumonia 15% (8-23), Haemophilus influenzae 19% (8-33), Moraxella Catharalis 7% (0-19), Staphylococcus aureus 7% (3-12), Mycobacterial infection 1% (0-2), Pneumocystis Jiroveci 1% (0-2), Pseudomonas 6% (2-10), Aspergillus 3% (1-5), Enterobacteriaceae 6% (2-13), Mycoplasma 2% (0-4); [e]Not only pulmonary; [f]Giardia intestintalis 11% (3-22),

Overall		General population prevalence
Pooled proportion (95% CI)	**I^2**	**Lifetime prevalence percentage estimates from various sources**
13 (7-22)	87	n/a
12 (9-16)	79	n/a
2 (1-3)	44	n/a
1 (1-2)	48	0.048% (1)
n/a	n/a	n/a

Salmonella species 6% (3-11);[g] Giardia intestinalis 13% (7-21), Salmonella species 6% (2-12), Campylobacter species 4% (1-8), Clostridium difficile 2% (1-3), cytomegalovirus 2% (0-7), Cryptosporidium species 1% (0-2), Helicobacter pylori 9% (3-18), Escherichia coli 8% (4-13), Candida species 10% (4-19), Strongyloidiasis 7% (3-13);[h] Histologic findings of villous atrophy are similar to those found in patients with celiac disease, but with some differences; in CVID plasma cells are absent from the intestinal lamina propria, and the crypt epithelium is not hyperplastic;[i] Prevalence, not lifetime prevalence;[j] Streptococcus pneumonia 3% (2-6), Haemophilus influenzae 1% (0-3);[k] Streptococcus pneumonia 3% (2-6), Haemophilus influenzae 1% (0-3).

Abbreviations: COPD, chronic obstructive pulmonary disease; GLILD, Granulomatous-lymphocytic interstitial lung disease; n/a, not applicable.

REFERENCES

1. Eaton WW, Pedersen MG, Atladóttir HO, Gregory PE, Rose NR, Mortensen PB. The prevalence of 30 ICD-10 autoimmune diseases in Denmark. Immunol Res. 2010 Jul;47(1–3):228–31.
2. https://seer.cancer.gov/statfacts/html/all.html.
3. https://ftp.cdc.gov/pub/Health_Statistics/NCHS/NHIS/SHS/2018_SHS_Table_A-2.pdf.
4. https://www.erswhitebook.org/chapters/bronchiectasis/.
5. Ghouri N, Hippisley-Cox J, Newton J, Sheikh A. Trends in the epidemiology and prescribing of medication for allergic rhinitis in England. J R Soc Med. 2008 Sep;101(9):466–72.
6. Ley B, Collard HR. Epidemiology of idiopathic pulmonary fibrosis. Clin Epidemiol. 2013 Nov;5:483–92.
7. Simpson CR, Newton J, Hippisley-Cox J, Sheikh A. Incidence and prevalence of multiple allergic disorders recorded in a national primary care database. J R Soc Med. 2008 Nov;101(11):558–63.
8. Thompson PL, Gilbert RE, Long PF, Saxena S, Sharland M, Wong ICK. Effect of antibiotics for otitis media on mastoiditis in children: a retrospective cohort study using the United kingdom general practice research database. Pediatrics. 2009 Feb;123(2):424–30.
9. Simpson CR, Hippisley-Cox J, Sheikh A. Trends in the epidemiology of chronic obstructive pulmonary disease in England: a national study of 51 804 patients. Br J Gen Pract J R Coll Gen Pract. 2010 Jul;60(576):277–84.
10. Arasaradnam RP, Brown S, Forbes A, Fox MR, Hungin P, Kelman L, et al. Guidelines for the investigation of chronic diarrhoea in adults: British Society of Gastroenterology, 3rd edition. Gut. 2018 Aug;67(8):1380–99.
11. Shaheen NJ, Mukkada V, Eichinger CS, Schofield H, Todorova L, Falk GW. Natural history of eosinophilic esophagitis: a systematic review of epidemiology and disease course. Dis esophagus Off J Int Soc Dis Esophagus. 2018 Aug;31(8).
12. Rodriguez-Castro KI, Franceschi M, Noto A, Miraglia C, Nouvenne A, Leandro G, et al. Clinical manifestations of chronic atrophic gastritis. Acta Biomed. 2018 Dec;89(8-S):88–92.
13. Nagra N, Dang S. Protein Losing Enteropathy. In Treasure Island (FL); 2020.
14. Wang H-Y, Leena KB, Plymoth A, Hergens M-P, Yin L, Shenoy KT, et al. Prevalence of gastro-esophageal reflux disease and its risk factors in a community-based population in southern India. BMC Gastroenterol. 2016 Mar;16:36.
15. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Dis Mon. 2003 Feb;49(2):53–70.
16. Farruggia P, Dufour C. Diagnosis and management of primary autoimmune neutropenia in children: insights for clinicians. Ther Adv Hematol. 2015 Feb;6(1):15–24.
17. Jaime-Pérez JC, Aguilar-Calderón PE, Salazar-Cavazos L, Gómez-Almaguer D. Evans syndrome: clinical perspectives, biological insights and treatment modalities. J Blood Med. 2018;9:171–84.
18. Lopez A, Cacoub P, Macdougall IC, Peyrin-Biroulet L. Iron deficiency anaemia. Lancet (London, England). 2016 Feb;387(10021):907–16.
19. Laupland KB, Gregson DB, Zygun DA, Doig CJ, Mortis G, Church DL. Severe bloodstream infections: a population-based assessment. Crit Care Med. 2004 Apr;32(4):992–7.
20. https://ftp.cdc.gov/pub/Health_Statistics/NCHS/NHIS/SHS/2018_SHS_Table_A-5.pdf.
21. McGill F, Griffiths MJ, Bonnett LJ, Geretti AM, Michael BD, Beeching NJ, et al. Incidence, aetiology, and sequelae of viral meningitis in UK adults: a multicentre prospective observational cohort study. Lancet Infect Dis. 2018 Sep;18(9):992–1003.

22. Savettieri G, Salemi G, Rocca WA, Meneghini F, Santangelo R, Morgante L, et al. Incidence and lifetime prevalence of Bell's palsy in two Sicilian municipalities. Sicilian Neuro-Epidemiologic Study (SNES) Group. Acta Neurol Scand. 1996 Jul;94(1):71–5.
23. Hanewinckel R, van Oijen M, Ikram MA, van Doorn PA. The epidemiology and risk factors of chronic polyneuropathy. Eur J Epidemiol. 2016 Jan;31(1):5–20.
24. https://www.who.int/news-room/fact-sheets/detail/epilepsy.
25. McBride S, Mowbray J, Caughey W, Wong E, Luey C, Siddiqui A, et al. Epidemiology, Management, and Outcomes of Large and Small Native Joint Septic Arthritis in Adults. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2020 Jan;70(2):271–9.
26. https://ftp.cdc.gov/pub/Health_Statistics/NCHS/NHIS/SHS/2018_SHS_Table_A-4.pdf.
27. Kremers HM, Nwojo ME, Ransom JE, Wood-Wentz CM, Melton LJ 3rd, Huddleston PM 3rd. Trends in the epidemiology of osteomyelitis: a population-based study, 1969 to 2009. J Bone Joint Surg Am. 2015 May;97(10):837–45.
28. https://www.iofbonehealth.org/facts-statistics.
29. https://www-uptodate-com.ru.idm.oclc.org/contents/peripheral-lymphadenopathy-in-children-etiology?search=Peripheral%20lymphadenopathy%20in%20children:%20Etiology&source=search_result&selectedTitle=1~150&usage_type=default&display_rank=1.
30. Chapman J, Bansal P, Goyal A, Azevedo AM. Splenomegaly. In Treasure Island (FL); 2020.
31. https://www.thyroid.org/media-main/press-room/.
32. Barbarot S, Auziere S, Gadkari A, Girolomoni G, Puig L, Simpson EL, et al. Epidemiology of atopic dermatitis in adults: Results from an international survey. Allergy. 2018 Jun;73(6):1284–93.
33. https://www.skincancer.org.
34. Bowsher D. The lifetime occurrence of Herpes zoster and prevalence of post-herpetic neuralgia: A retrospective survey in an elderly population. Eur J Pain. 1999 Dec;3(4):335–42.
35. Malvy D, Ezzedine K, Lançon F, Halioua B, Rezvani A, Bertrais S, et al. Epidemiology of orofacial herpes simplex virus infections in the general population in France: results of the HERPIMAX study. J Eur Acad Dermatol Venereol. 2007 Nov;21(10):1398–403.
36. Jacob L, John M, Kalder M, Kostev K. Prevalence of vulvovaginal candidiasis in gynecological practices in Germany: A retrospective study of 954,186 patients. Curr Med Mycol. 2018 Mar;4(1):6–11.
37. Torsten Zuberbier, Clive Grattan MM. Urticaria and Angioedema. 2010.
38. Zain RB. Oral recurrent aphthous ulcers/stomatitis: prevalence in Malaysia and an epidemiological update. J Oral Sci. 2000 Mar;42(1):15–9.
39. Chapel H, Lucas M, Lee M, Bjorkander J, Webster D, Grimbacher B, et al. Common variable immunodeficiency disorders: division into distinct clinical phenotypes. Blood. 2008 Jul;112(2):277–86.

Supplementary Table 5.5. Clinical manifestations in patients with common variable immunodeficiency[a]

Manifestation	Pooled proportion (95% CI)	Nr. of participants	Nr. of studies
Clinical phenotype[b]			
Infections only	48 (39-58)	1677	16
Autoimmunity	27 (22-32)	4061	28
Polyclonal lymphocytic infiltration	29 (22-37)	2252	9
Enteropathy	9 (6-13)	2400	10
Lymphoid malignancy	5 (3-7)	3392	20
Malignancy	10 (7-14)	2289	25
Respiratory involvement			
Hypersensitivity pneumonitis	1 (n/a)	69	1
Otitis	43 (35-51)	11444	18
Sinusitis	67 (57-77)	1887	21
Upper respiratory tract infections	84 (75-92)	794	15
Pneumonia	62 (54-70)[c]	1876	22
Bronchitis	62 (44-78)	1166	8
Lower respiratory tract infections	78 (64-90)	270	8
Bronchiectasis	32 (26-38)	3720	31
Lymphocytic interstitial pneumonia	3 (1-5)	928	5
GLILD	15 (7-25)	2094	11
Lymphoid hyperplasia[d]	10 (5-17)	1667	11
Follicular bronchiolitis	1 (n/a/)	69	1
Asthma	25 (17-35)	1819	15
Allergic rhinitis	18 (8-31)	1282	7
Idiopathic lung fibrosis	12 (1-30)	247	6
Emphysema	4 (1-10)	79	2
Pulmonary lobe resection	5 (3-8)	285	6
Cryptococcal lung abscess	0 (n/a/)	248	1
Respiratory tract infections	92 (78-99)	543	5
Lung cancer	1 (0-1)	1993	4
Chronic lung disease	44 (27-63)	716	3
Allergy	33 (13-56)	419	6
Mastoiditis	3 (1-7)	532	3
Interstitial lung disease	13 (6-23)	629	4
Lung nodules	3 (1-6)	532	2
Pharyngitis/tonsillitis	18 (12-24)	575	3
Conjunctivitis	10 (5-16)	872	6

Supplementary Table 5.5. Continued.

Manifestation	Pooled proportion (95% CI)	Nr. of participants	Nr. of studies
COPD	34 (14-58)	51	2
Pulmonary hypertension	3 (n/a)	988	1
Sino nasal polyps	5 (n/a)	988	1
Lung transplantations	1 (n/a)	473	1
Rhino-pharyngeal cancer	1 (n/a)	75	1
Bronchiolitis obliterans	4 (n/a)	69	1
Sarcoidosis	2 (n/a)	988	1
Gastrointestinal involvement			
Food intolerance	19 (n/a)	32	1
Chronic diarrhoea of unknown origin	27 (21-34)	1885	14
Gastrointestinal tract infection	29 (21-38)[e]	1566	14
Intestinal granulomatosis	1 (0-4)	980	4
Inflammatory bowel disease	10 (6-16)	1940	14
Crohn's disease	3 (2-4)	703	5
Ulcerative colitis	2 (1-3)	861	7
Celiac disease	3 (2-4)	1586	9
Atrophic gastritis	5 (3-9)	652	7
Stomach cancer	2 (1-4)	1754	11
Colorectal cancer	1 (0-2)	1318	6
Eosinophilic inflammation	5 (2-10)	121	3
Protein losing enteropathy	1 (0-3)	293	2
Gastritis	28 (22-35)	286	3
Peritonitis	4 (1-8)	132	3
Villous atrophy	11 (1-28)	291	2
Malabsorption	13 (4-26)	1099	5
Autoimmune gastro-intestinal disease	13 (5-24)	1083	2
Gastro-oesophageal reflux disease	16 (8-25)	565	3
Lymphocytic colitis	9 (4-16)	92	2
Chronic appendicitis	1 (n/a)	95	1
Erythema nodosum	3 (n/a)	95	1
Gastrointestinal adenocarcinoma	2 (n/a)	47	1
Primary sclerosing cholangitis	4 (n/a)	23	1
Increased intraepithelial lymphocytes	60 (n/a)	53	1
Gastric metaplasia	25 (n/a)	53	1
Stomatitis	5 (n/a)	43	1
Oesophageal cancer	0 (n/a)	473	1

Supplementary Table 5.5. Continued.

Manifestation	Pooled proportion (95% CI)	Nr. of participants	Nr. of studies
Ulcerative proctitis	1 (n/a)	248	1
Sprue-like disease (intestines)	2 (n/a)	248	1
Glandular tissue			
Sjogren's syndrome	3 (1-6)	1736	7
Breast cancer	1 (1-2)	2079	8
Cervix cancer	1 (1-2)	1171	3
Uterine cancer	1 (0-2)	679	2
Hepatic involvement			
Autoimmune hepatitis	4 (0-10)	1337	5
Primary biliary cirrhosis	2 (1-3)	1011	2
Unexplained hepatomegaly	14 (8-72)	1507	8
Viral hepatitis	4 (2-6)	1194	6
Liver granuloma	3 (1-6)	1058	5
Liver cirrhosis	4 (1-9)	92	2
Liver cancer	1 (0-1)	928	2
Hepatitis (not further specified)	4 (0-12)	816	3
Liver abscess	1 (n/a)	69	1
Autoimmune liver disease	8 (n/a)	988	1
Nodular regenerative hyperplasia	1 (n/a)	988	1
Urinary tract involvement			
Urinary tract infection	14 (11-18)	1267	12
Pyelonephritis	8 (0-22)	205	2
Granuloma in kidney	0 (n/a)	455	1
Genitourinary cancer	3 (n/a)	455	1
Prostatic cancer	1 (n/a)	455	1
Nephrotic syndrome	1 (n/a)	248	1
Hematologic involvement			
Idiopathic thrombocytopenia	10 (7-14)	2717	18
Autoimmune cytopenia	13 (10-17)	3131	17
Autoimmune haemolytic anaemia	6 (4-8)	2895	20
Autoimmune neutropenia	4 (2-6)	1944	9
Unknown/other neutropenia	6 (3-10)	1653	8
Evans syndrome	2 (1-2)	1208	3
Polycythaemia	0 (n/a)	224	1
Leukopenia	9 (n/a)	77	1
Haemolytic anaemia	5 (n/a)	990	1

Supplementary Table 5.5. Continued.

Manifestation	Pooled proportion (95% CI)	Nr. of participants	Nr. of studies
Iron deficiency anaemia	10 (2-22)	1322	2
Pernicious anaemia	3 (1-5)	2142	10
Bloodstream infection	9 (5-13)	1872	16
Central-line associated blood stream infection	0 (n/a)	457	1
Unknown/other anaemia	10 (n/a)	988	1
Unknown/other thrombocytopenia	8 (n/a)	988	1
Lymphopenia	9 (n/a)	988	1
Endocarditis	2 (n/a)	43	1
Wegener's granulomatosis	2 (n/a)	45	1
Myelodysplasia	0 (n/a)	988	1
Neurological involvement			
Infectious meningitis	7 (5-9)[f]	1672	17
Brain abscess	2 (n/a)	43	1
Progressive multifocal leukoencephalopathy	0 (n/a)	32	1
Granuloma in brain	0 (n/a)	473	1
Myasthenia gravis	0 (0-1)	1520	3
Multiple sclerosis	0 (0-1)	1022	2
Bell's palsy	1 (0-2)	1000	2
Polyneuropathy	3 (1-6)	1011	2
Epilepsy	6 (2-11)	480	2
Brain cancer	2 (n/a)	47	1
Meningioma	1 (n/a)	75	1
Uveitis	2 (n/a)	988	1
Granuloma in retina	0 (n/a)	455	1
Attention deficit hyperactivity disorder	6 (n/a)	457	1
Anxiety	3 (n/a)	457	1
Developmental delay	7 (n/a)	457	1
Headaches/migraine	17 (n/a)	457	1
Cerebral atrophy	1 (n/a)	248	1
Schizophrenia	1 (n/a)	248	1
Bone and joint involvement			
Septic arthritis	4 (1-8)	719	7
Rheumatoid arthritis	3 (2-4)	1922	8
Seronegative arthritis	4 (2-7)	1284	7
Juvenile idiopathic arthritis	1 (0-4)	1160	3

Supplementary Table 5.5. Continued.

Manifestation	Pooled proportion (95% CI)	Nr. of participants	Nr. of studies
Psoriatic arthritis	0 (0-1)	1022	2
Arthralgia	20 (n/a)	457	1
Osteomyelitis	4 (1-8)	807	6
Multiple myeloma	0 (0-1)	1212	2
Ewing sarcoma	0 (n/a)	224	1
Kaposi's sarcoma	0 (0-1)	707	2
Osteoporosis/osteopenia	10 (1-25)	480	2
Fasciitis	2 (n/a)	43	1
Myeloid sarcoma	1 (n/a)	106	1
Myositis	1 (n/a)	988	1
Mixed connective tissue disease	1 (n/a)	988	1
Scleroderma/CREST	0 (n/a)	988	1
Nasopharyngeal soft tissue sarcoma	8 (n/a)	12	1
Osteochondroma	5 (n/a)	22	1
Polymyalgia rheumatica	3 (n/a)	32	1
Psoas abscess	0 (n/a)	248	1
Sacroiliitis	1 (n/a)	95	1
Lymph node and spleen involvement			
Lymphadenopathy	30 (20-42)	2122	13
Non-malignant lymphoproliferation	28 (16-40)	1462	6
Splenomegaly	29 (22-37)	3153	22
Non-Hodgkin's lymphoma	4 (3-5)	2698	13
Splenectomy	6 (3-8)	1068	5
Pancreatic cancer	2 (0-7)	478	2
Leukaemia	1 (1-2)	1581	4
Granuloma in lymph node	2 (0-5)	928	2
Granuloma in spleen	1 (0-2)	928	2
Granuloma in bone marrow	0 (0-1)	928	2
Waldenstrom's macroglobulinemia	0 (n/a)	248	1
Endocrinological involvement			
Thyroid disease (all)	9 (4-16)	2252	14
Autoimmune thyroiditis	4 (2-6)	2215	11
Diabetes mellitus	2 (1-3)	1523	8
Thyroid cancer	1 (0-3)	951	3
Addison-Biermer disease	1 (n/a)	77	11
Pituitary gland adenoma	1 (n/a)	77	1

Supplementary Table 5.5. Continued.

Manifestation	Pooled proportion (95% CI)	Nr. of participants	Nr. of studies
Adrenal tumour	1 (n/a)	71	1
Growth hormone deficiency	25 (n/a)	12	1
Ovarian cancer	0 (n/a)	473	1
Muco/cutaneous involvement			
Psoriasis	3 (2-5)	1679	6
Atopic dermatitis	12 (7-18)	1307	6
Alopecia	3 (2-4)	1425	6
Vitiligo	5 (3-8)	2440	12
Warts	5 (4-7)	825	4
Skin cancer	3 (1-5)	1319	7
Skin infections	13 (10-17)	1033	11
Cutaneous abscesses	5 (2-10)	72	6
(Recurrent) herpes zoster	9 (5-14)	1178	9
(Recurrent) herpes simplex	9 (2-10)	404	7
Oral candidiasis	6 (2-11)	1514	9
Genital candidiasis	6 (3-12)	114	2
Urticaria	5 (3-8)	1299	5
Oral/dental infections	4 (3-6)	489	2
Skin granuloma	1 (0-2)	928	2
Severe varicella	4 (2-7)	284	2
Recurrent parotitis	6 (0-3)	279	2
Clubbing	19 (0-73)	138	2
Aphthous lesions	16 (5-31)	147	3
Lichen planus	2 (n/a)	47	1
Neutrophilic dermatosis	23 (n/a)	31	1
Mastocytosis	0 (n/a)	988	1
Angioedema	4 (n/a)	23	1
Behcet's disease	2 (n/a)	43	1
Granulomatous gingival hyperplasia	2 (n/a)	126	1
Granuloma in retina	1 (n/a)	77	1
Oral cancer	0 (n/a)	473	1
Vaginal cancer	0 (n/a)	473	1
Other clinical manifestations			
Growth retardation/weight loss	13 (7-22)	759	8
Unexplained granuloma	12 (9-16)	2348	11
Fatigue	39 (n/a)	457	1

Supplementary Table 5.5. Continued.

Manifestation	Pooled proportion (95% CI)	Nr. of participants	Nr. of studies
Vasculitis	2 (1-3)	1782	6
Systemic lupus erythematosus	1 (1-2)	2288	8

Supplementary Table 5.5. [a]Pathogens were only included in this table if the pathogen was mentioned in ≥2 studies; [b]According to Chapel et al; [c]Streptococcus pneumonia 15% (8-23), Haemophilus influenzae 19% (8-33), Moraxella Catharalis 7% (0-19), Staphylococcus aureus 7% (3-12), Mycobacterial infection 1% (0-2), Pneumocystis Jiroveci 1% (0-2), Pseudomonas 6% (2-10), Aspergillus 3% (1-5), Enterobacteriaceae 6% (2-13), Mycoplasma 2% (0-4); [d]Not only pulmonary; [e]Giardia intestinalis 13% (7-21), Salmonella species 6% (2-12), Campylobacter species 4% (1-8), Clostridium difficile 2% (1-3), cytomegalovirus 2% (0-7), Cryptosporidium species 1% (0-2), Helicobacter pylori 9% (3-18), Escherichia coli 8% (4-13), Candida species 10% (4-19), Strongyloidiasis 7% (3-13); [f]Streptococcus pneumonia 3% (2-6), Haemophilus influenzae 1% (0-3).

Abbreviations: COPD, chronic obstructive pulmonary disease; CREST syndrome, calcinosis, Raynaud's phenomenon, oesophageal dysmotility, sclerodactyly, telangiectasia; GLILD, Granulomatous-lymphocytic interstitial lung disease; n/a, not applicable.

Supplementary Table 5.6. Subgroup analyses of clinical manifestations in children versus adults.

	Children		Adults	
Manifestation	**Pooled proportion (95% CI)**	**I^2**	**Pooled proportion (95% CI)**	**I^2**
Clinical phenotype[a]				
Infections only	76 (57-91)	84	38 (27-49)	70
Autoimmunity	14 (5-27)	90	21 (12-30)	94
Polyclonal lymphocytic infiltration	n/a	n/a	25 (21-29)	0
Enteropathy	10 (3-20)	78	14 (10-18)	67
Lymphoid malignancy	5 (2-10)	55	6 (5-8)	0
Malignancy	5 (3-7)	0	16 (11-21)	85
Respiratory involvement				
Otitis	55 (45-64)	68	32 (27-37)	42
Sinusitis	62 (50-74)	79	74 (60-86)	91
Upper respiratory tract infection	69 (45-89)	91	79 (66-90)	80
Pneumonia	61 (50-71)[b]	74	66 (51-79)[c]	93
Bronchitis	59 (11-71)	97	72 (38-97)	98
Lower respiratory tract infection	71 (59-82)	0	n/a	n/a
Bronchiectasis	16 (9-25)	81	36 (27-46)	93
GLILD	6 (4-9)	0	33 (13-57)	97
Lymphoid hyperplasia	2 (0-6)	30	11 (2-24)	94
Asthma	3 (5-9)	93	33 (21-47)	94

Supplementary Table 5.6. Continued.

	Children		Adults	
Manifestation	**Pooled proportion (95% CI)**	**I^2**	**Pooled proportion (95% CI)**	**I^2**
Allergic rhinitis	17 (3-39)	83	n/a	n/a
Allergy	49 (33-65)	40	n/a	n/a
Mastoiditis	4 (0-15)	81	n/a	n/a
Lung cancer	n/a	n/a	1 (0-2)	46
Interstitial lung disease	6 (1-14)	51	15 (3-35)	90
Pharyngitis/tonsillitis	17 (8-28)	78	n/a	n/a
Conjunctivitis	16 (8-25)	64	n/a	n/a
Gastrointestinal involvement				
Chronic diarrhoea	17 (11-24)	50	34 (22-48)	93
Gastrointestinal tract infection	35 (18-55)[d]	89	34 (8-65)[e]	98
Inflammatory bowel disease	3 (1-6)	32	18 (6-33)	96
Celiac disease	3 (1-5)	10	2 (1-4)	32
Atrophic gastritis	n/a	n/a	9 (0-26)	87
Stomach cancer	n/a	n/a	2 (0-6)	82
Colorectal cancer	n/a	n/a	1 (0-4)	75
Malabsorption	5 (3-8)	0	11 (0-32)	97
Gastro-oesophageal reflux disease	12 (1-30)	90	n/a	n/a
Glandular tissue				
Sjogren's syndrome	n/a	n/a	3 (0-8)	82
Breast cancer	n/a	n/a	2 (1-3)	49
Hepatic involvement				
Autoimmune hepatitis	2 (0-6)	42	4 (0-12)	92
Viral hepatitis	n/a	n/a	2 (1-5)	69
Liver cancer	n/a	n/a	1 (0-1)	44
Unexplained hepatomegaly	21 (2-50)	96	n/a	n/a
Urinary tract involvement				
Urinary tract infection	11 (8-15)	0	15 (12-19)	0
Pyelonephritis	8 (0-22)	70	n/a	n/a
Hematologic involvement				
Idiopathic thrombocytopenia	9 (2-21)	87	6 (2-12)	86
Autoimmune cytopenia	15 (4-30)	76	16 (7-27)	67
Autoimmune haemolytic anaemia	6 (4-9)	0	5 (2-8)	68
Autoimmune neutropenia	4 (0-11)	83	2 (0-6)	86
Evans syndrome	9 (0-26)	86	n/a	n/a
Pernicious anaemia	1 (0-3)	61	1 (1-3)	29

Supplementary Table 5.6. Continued.

	Children		Adults	
Manifestation	**Pooled proportion (95% CI)**	**I^2**	**Pooled proportion (95% CI)**	**I^2**
Bloodstream infection	8 (5-12)	29	12 (9-16)	47
Neurologic involvement				
Infectious meningitis	7 (2-13)	69	7 (4-9)	29
Bell's palsy	2 (0-17)	78	n/a	n/a
Multiple sclerosis	n/a	n/a	1 (0-1)	6
Bone and joint involvement				
Septic arthritis	8 (1-22)	67	n/a	n/a
Rheumatoid arthritis	n/a	n/a	4 (1-7)	64
Seronegative arthritis	2 (0-6)	55	n/a	n/a
Psoriatic arthritis	n/a	n/a	1 (0-2)	57
Osteomyelitis	2 (0-6)	68	n/a	n/a
Kaposi's sarcoma	n/a	n/a	0 (0-1)	0
Lymph node and spleen involvement				
Lymphadenopathy	22 (44-48)	96	n/a	n/a
Non-malignant lymphoproliferation	n/a	n/a	27 (12-46)	89
Splenomegaly	20 (6-39)	94	30 (16-46)	95
Non-Hodgkin's lymphoma	1 (0-3)	14	5 (4-6)	40
Hodgkin's lymphoma	5 (2-11)	0	1 (0-2)	0
Splenectomy	n/a	n/a	7 (5-10)	17
Leukaemia	n/a	n/a	1 (0-1)	16
Endocrinological involvement				
Thyroid disease	8 (0-24)	66	29 (12-50)	96
Autoimmune thyroiditis	3 (1-6)	0	6 (2-12)	83
Diabetes mellitus	2 (0-4)	0	4 (0-14)	82
Thyroid cancer	n/a	n/a	1 (0-2)	74
Muco/cutaneous involvement				
Psoriasis	1 (0-6)	72	4 (2-7)	49
Atopic dermatitis	16 (12-20)	0	n/a	n/a
Alopecia	1 (0-3)	0	n/a	n/a
Vitiligo	2 (1-4)	0	2 (1-4)	48
Warts	6 (3-9)	0	5 (3-7)	0
Skin cancer	1 (0-3)	28	5 (2-8)	64
Skin infections	16 (9-25)	64	13 (6-23)	73
Recurrent herpes zoster infections	19 (12-27)	0	7 (2-15)	91
Recurrent herpes simplex infections	n/a	n/a	17 (0-47)	96

Supplementary Table 5.6. Continued.

	Children		Adults	
Manifestation	**Pooled proportion (95% CI)**	**I^2**	**Pooled proportion (95% CI)**	**I^2**
Oral candidiasis	12 (8-16)	2	6 (0-20)	96
Urticaria	3 (1-6)	28	n/a	n/a
Clubbing	5 (1-12)	49	n/a	n/a
Other clinical manifestations				
Growth retardation	19 (15-23)	0	n/a	n/a
Unexplained granuloma	6 (2-12)	53	15 (7-25)	90
Vasculitis	1 (0-5)	47	2 (2-4)	0
Systemic lupus erythematosus	1 (0-9)	75	2 (1-3)	0

Supplementary Table 5.6. [a]According to Chapel et al; [b]Streptococcus pneumoniae 10% (0-29); [c]Streptococcus pneumoniae 10% (0-28), Haemophilus influenzae 8% (4-14), Mycobacterial infection 1% (0-2), Pneumocystis Jiruveci 1% (0-2), Pseudomonas 2% (1-5); [d]Giardia intestinalis 13% (7-20), Salmonella species 8% (2-18), Campylobacter species 5% (0-14).; [e]Giardia intestinalis 12% (0-32), Salmonella species 3% (0-12), Campylobacter species 3% (0-10), Clostridium difficile 2% (0-3), Cryptosporidium species 1% (0-2), Helicobacter pylori 6% (1-12).

Chapter 6

Protocol for the unclassified primary antibody deficiency (unPAD) study: characterization and classification of patients using the ESID online Registry

Lisanne M.A. Janssen, MD, Ineke C.G.M. Reijnen, MD, Cinzia Milito, MD, PhD, David Edgar, MD, PhD, Helen Chapel, MD, PhD, Esther de Vries, MD, PhD, and the unPAD consortium

PLoS One. 2022; 17(3):e0266083

ABSTRACT

Background

Primary antibody deficiencies (PADs) without an identified monogenetic origin form the largest and most heterogeneous group of primary immunodeficiencies. These patients often remain undiagnosed for years and many present to medical attention in adulthood after several infections risking structural complications. Not much is known about their treatment, comorbidities, or prognosis, nor whether the various immunological forms (decreased total IgG, IgG subclass(es), IgM, IgA, specific antibody responses, alone or in combination(s)) should be considered as separate, clearly definable subgroups. The unclassified primary antibody deficiency (unPAD) study aims to describe in detail all PAD patients *without* an identified specific monogenetic defect regarding their demographical, clinical, and immunological characteristics at presentation and during follow-up. In constructing these patterns, the unPAD study aims to reduce the number of missed and unidentified PAD patients in the future. In addition, this study will focus on subclassifying unPAD to support the identification of patients at higher risk for infection or immune dysregulation related complications, enabling the development of personalized follow-up and treatment plans.

Methods and analysis

We present a protocol for a multicenter observational cohort study using the ESID online Registry. Patients of all ages who have given informed consent for participation in the ESID online Registry and fulfill the ESID Clinical Working Definitions for 'unclassified antibody deficiency', 'deficiency of specific IgG', 'IgA with IgG subclass deficiency', 'isolated IgG subclass deficiency', 'selective IgM deficiency', 'selective IgA deficiency' or 'common variable immunodeficiency' will be included. For all patients, basic characteristics can be registered at first registration and yearly thereafter in level 1 forms. Detailed characteristics of the patients can be registered in level 2 forms. Consecutive follow-up forms can be added indefinitely. To ensure the quality of the collected data, all data will be fully monitored before they are exported from the ESID online Registry for analysis. Outcomes will be the clinical and immunological characteristics of unPAD at presentation and during follow-up. Subgroup analyses will be made based on demographical, clinical and immunological characteristics.

INTRODUCTION

Ear-nose-throat (ENT) and lower airway symptoms occur commonly in the general population; they are often, but not always, caused by infection. These infections already start early in life, are mostly viral in origin and self-limiting. When symptoms continue to recur, allergy, asthma, smoking and/or (in adults) chronic obstructive pulmonary disease (COPD) can be the underlying cause [1]. Only a small number of patients suffer from too many, too frequent, unusual and/or severe infections caused by inborn errors of immunity (IEI). The majority of IEI patients suffer from predominantly antibody deficiencies (PAD), which are generally not immediately life-threatening. PADs can be subdivided into the rare, more severe, agammaglobulinemias and hyper-IgM syndromes, and the less rare hypogammaglobulinemias [2]. The latter may remain undiagnosed for years [2–5]; however, also these can ultimately lead to important morbidity, irreversible organ damage and reduced lifespan when they are not recognized and adequately treated in time [6–8].

Traditionally, common variable immunodeficiency disorders (CVID) are considered a separate PAD entity, comprising the most severe hypogammaglobulinemia patients [9,10]. CVID is the most common form seen in specialized centers (estimated prevalence in the population 1: 10.000-50.000) [11]. However, even for CVID, expert opinion varies as to which patients with decreased IgG and disturbed specific antibody responses should be classified under this diagnosis, some considering combination with decreased IgA *or* decreased IgM sufficient, and others diagnosing CVID *only* in case IgA is decreased (± decreased IgM) [12]. Many more patients suffer from less-well described and understood forms of hypogammaglobulinemia: decreased total IgG, IgG-subclass(es), IgM, IgA and/or specific antibodies, alone, or in combination(s) [2]. The International Union of Immunological Societies (IUIS) has grouped these cases together in the 'predominantly antibody deficiencies' section as 'isotype/light chain/functional deficiencies' (with a subdivision based on immunological laboratory values; **Table 6.1**) [13]; in the European Society for Immunodeficiencies (ESID) Clinical Working Definitions they are divided in separate entities which overlap in part with the IUIS subdivisions (**Table 6.2**) [14]. However, these PAD cases are often difficult to classify, either because aspects of more than one subgroup are found within the same patient, or because the patient's immune capacity has not been sufficiently investigated to be positioned in a specific subgroup. They are therefore often referred to as "other hypogammaglobulinemia" or - more recently - as "unclassified primary antibody deficiency (unPAD)" [15]. Within this group, clinical severity as well as the results of immunological laboratory investigations and potential underlying pathophysiology may differ greatly. Also, different centers are inclined to treat the classification of these patients in different ways, making comparative studies difficult to perform.

Because IEI are rare disorders, international collaboration is necessary to study these diseases. Since 2004, the ESID has been running an online database for primary immunodeficiencies: the ESID online Registry [16]. This database currently comprises information on more than 30,000 patients with errors of immunity. Documentation is organized in different levels. Level 1 is a basic dataset comprising the IEI diagnosis, demographic data, the way to diagnosis (including the presenting symptoms), immunoglobulin replacement therapy, hematopoietic stem cell transplantation and gene therapy. This level 1 information is meant for documentation of all patients who gave informed consent, with yearly concise follow-up documentation. An additional level 2 form was developed for more extensive long-term documentation of hypogammaglobulinemia patients which comprises a comprehensive dataset with additional items: additional clinical features, current and previous medications, diagnostic vaccinations, virological analyses, instrumental data (lung function, chest HRCT and gastroscopy), blood cell count, immunoglobulins, lymphocyte subsets, auto-antibodies, and further details on therapy.

Because of the moderately decreased immunoglobulin levels, unPADs are often considered to be clinically milder. However, unPAD-related symptoms can lead to decreased quality of life, loss of participation in society (school, work) and higher health care costs [6–8,17–19]. These people are often not recognized as IEI patients, because the general public as well as most health care professionals - who are not specialized in immunodeficiency - do not consider IEI in people with recurrent 'normal' infections. The concomitant fatigue these patients suffer is often considered to be of psychosocial origin or is interpreted as 'chronic fatigue syndrome'.

We therefore initiated the unPAD study, based on the ESID online Registry, to describe in detail all types of PAD patients *without* an identified specific monogenetic origin (thus excluding e.g. X-linked and autosomal recessive agammaglobulinemia, and class-switch recombination defects) regarding their demographical, clinical and immunological characteristics at presentation and during follow-up, and to identify subgroups based upon the patterns in these characteristics which can support refining of the classification. By better characterization and classification of the disease, the unPAD study aims to support reducing the number of missed and unidentified PAD patients in the future. To ensure the quality of the collected data, all data will be fully monitored before they are exported from the ESID online Registry system for analysis. In this article, we describe in detail the design of the unPAD study, including the strict monitoring rules, and the planned statistical analysis of the obtained data.

Table 6.1. IUIS phenotypical classification – predominantly antibody deficiencies (without an identified monogenetic origin).

Phenotypical classification	Criteria
Hypogammaglobulinemia	
Common variable immunodeficiency (CVID) Phenotype (with no known disease-causing monogenic defect specified)	Decrease of IgG, IgA and/or IgM AND secondary causes of hypogammaglobulinemia have been excluded AND B cells > 1% Clinical phenotypes vary: most have recurrent infections, some have polyclonal lymphoproliferation, autoimmune cytopenias and/or granulomatous disease
Other antibody deficiencies	Isotype, light chain or functional deficiencies with generally normal numbers of B cells
IgG subclass deficiency with IgA deficiency	Recurrent bacterial infections May be asymptomatic Reduced IgA with decrease in one or more IgG subclass(es)
Isolated IgG subclass deficiency	Usually asymptomatic A minority may have poor antibody response to specific antigens and recurrent viral/bacterial infections Reduction in one or more IgG subclass(es)
Selective IgM deficiency	Pneumococcal/ bacterial infections Absent serum IgM
Selective IgA deficiency	May be asymptomatic Bacterial infections, autoimmunity mildly increased Very low to absent IgA with other isotypes normal, normal subclasses and specific antibodies
Specific antibody deficiency with normal immunoglobulin levels and normal B cells	Reduced ability to produce antibodies to specific antigens Immunoglobulin levels normal

Table 6.1. Source: Bousfiha et al. [13].

Table 6.2. The Clinical Working Definitions for predominantly antibody deficiencies in the ESID online Registry (without an identified monogenetic origin).

No.	Clinical Working Definition[a]	Criteria
1	**Common variable immunodeficiency disorders (CVID)**	Patients with at least one of the following: · Increased susceptibility to infection · Autoimmune manifestations · Unexplained granulomatous disease · Unexplained polyclonal lymphoproliferation · Affected family member with antibody deficiency AND marked decrease of IgG and marked decrease of IgA with or without low IgM levels (measured at least twice; <2SD of the normal levels for their age) AND at least one of the following: · Poor antibody response to vaccines (and/or absent Isohemagglutinins) · Low switched memory B cells (<70% of age-related normal value) AND secondary causes of hypogammaglobulinemia have been excluded AND diagnosis is established after the 4th year of life AND no evidence of profound T-cell deficiency, defined as 2 out of the following (y=year of life): · CD4 numbers/microliter: 2-6y <300, 6-12y <250, >12y <200 · % naïve CD4: 2-6y <25%, 6-16y <20%, >16 <10% · T cell proliferation absent
2	**Deficiency of specific IgG (specific antibody deficiency – SPAD)**	Infections (recurrent or severe bacterial) AND normal serum/plasma IgG, A and M and IgG subclass levels AND profound alteration of the antibody responses to S. pneumonia (or other polysaccharide vaccine) either after documented invasive infection or after test immunization AND exclusion of T-cell defect
3	**IgA with IgG subclass deficiency**	Infections (recurrent or severe bacterial) AND undetectable serum/plasma IgA level (with normal/lowish IgG and IgM levels) AND low levels in one of more IgG subclass (documented twice) AND normal IgG antibody response to some vaccinations AND exclusion of T-cell defect
4	**Isolated IgG subclass deficiency**	Infections (recurrent or severe bacterial) AND normal IgG, A and M serum/plasma levels AND low levels in 1, 2, 3 IgG subclass or several missing (documented twice) AND normal IgG antibody response to some vaccinations AND exclusion of T-cell defect

Table 6.2. Continued.

No.	Clinical Working Definition[a]	Criteria
5	**Selective IgM deficiency**	Infections (either invasive or recurrent, usually bacterial) AND low IgM serum/plasma level (with normal IgG and IgG subclasses and IgA plasma level) AND normal IgG antibody response to all vaccinations AND exclusion of T-cell defect
6	**Selective IgA deficiency**	At least one of the following: · Increased susceptibility to infection · Autoimmune manifestations · Affected family member AND diagnosis after 4th year of life AND undetectable serum IgA, but normal serum IgG and IgM (measured at least twice) AND secondary causes of hypogammaglobulinemia have been excluded AND normal IgG antibody response to vaccination AND exclusion of T-cell defect
7	**Unclassified antibody deficiency[b]**	Patients with at least 1 of the following 4: · Recurrent or severe bacterial infections · Autoimmune phenomena (especially cytopenia's) · Unexplained polyclonal lymphoproliferation · Affected family member AND at least one of the following: · Marked decrease of at least one of total IgG, IgG1, IgG2, IgG3, IgA or IgM levels · Failure of IgG antibody response(s) to vaccines AND secondary causes of hypogammaglobulinemia have been excluded (infection, protein loss, medication, pregnancy) AND no clinical signs of T-cell related disease AND does not fit any of the other working definitions (excluding 'unclassified immunodeficiencies')

Table 6.2. [a] For this project, the combined patients under working definitions 2-7 are referred to as 'unPAD patients'. [b] The criteria for working definitions 1-6 are very strict. All 'predominantly antibody deficiencies' that do not completely fulfil all criteria of any of these working definitions 1-6 should be registered under 7 - unclassified antibody deficiency. If the patient does not completely fulfil all criteria for 'unclassified antibody deficiency' he/she should be registered under 'unclassified immunodeficiency' (if applicable; it is also possible that no immunodeficiency whatsoever is present).
Source: https://esid.org/Working-Parties/Registry-Working-Party/Diagnosis-criteria.

METHODS

Study objective

F*or this project* the current Clinical Working Definitions in the ESID online Registry 'deficiency of specific IgG (specific antibody deficiency – SPAD)', 'IgA with IgG subclass deficiency', 'isolated IgG subclass deficiency', 'selective IgM deficiency', 'selective IgA deficiency', and 'unclassified primary antibody deficiency' [14] will hereafter be referred to as 'unPAD patients'. The unPAD study aims to characterize all types of PAD patients *without* an identified specific monogenetic origin, i.e. unPAD patients and patients fulfilling the Clinical Working Definition 'common variable immunodeficiency (CVID)' [14]. We will classify all included patients into subgroups with classification techniques using the demographical, clinical and/or immunological characteristics as directed by the best fit. Finally, we will analyze the predictive potential of demographical, clinical and/or immunological characteristics in relation to the occurrence of PAD-related complications such as bronchiectasis or cytopenias in both our newly defined hypogammaglobulinemia subgroups as well as in the subgroups based on the current Clinical Working Definitions.

Study questions underlying the level 2 ESID Registry variables

A subset of the members of the ESID Registry Working Party formulated the research questions underlying the unPAD level 2 forms of the ESID online Registry in (mainly remote) consensus discussions:

1. What is the clinical presentation of these patients *at diagnosis* (spectrum, observed prevalence, subgroups, age-related differences)?
2. What is the immunological presentation of these patients *at diagnosis*?
3. Can subgroups be identified *at diagnosis* based on clinical and/or immunological characteristics?
4. What is the clinical presentation of these patients *during follow-up* (spectrum, observed prevalence, subgroups, age-related differences)?
5. What is the immunological presentation of these patients *during follow-up*?
6. Can subgroups be identified based on clinical and/or immunological characteristics; if so, is this a stationary classification, or do patients develop from one subgroup to another / others *with time*?

And in the long run:

7. What is the prognosis of (subgroups of) these patients regarding infections, complications, long-term sequelae, life expectancy, quality of life and ability to function in society?

Patient eligibility

Before patient data can be entered into the ESID online Registry informed consent has to be obtained. The patient consent forms containing information on the ESID online Registry are available on the ESID website in many languages [20]. These forms need to be approved by a competent local Research Ethics Committee according to the regulations of the respective countries and documenting centers before use.

Inclusion criteria

1. The patient (or parents in case of children) has given informed consent for participation in the ESID online Registry.
2. The patient fulfils the ESID online Registry Clinical Working Definitions for 'unclassified antibody deficiency', 'deficiency of specific IgG (specific antibody deficiency – SPAD)', 'IgA with IgG subclass deficiency', 'isolated IgG subclass deficiency', 'selective IgM deficiency', 'selective IgA deficiency' or 'CVID' (specified in **Table 6.2**).
3. At least the registration set of both level 1 and level 2 'at diagnosis' forms has been completed.

Exclusion criteria

1. Refusal of the reporting physician to have all data that were entered by the center in the ESID online Registry checked and – if necessary – corrected under supervision of the unPAD study monitor(s).
2. Patients with an identified monogenetic disease-causing mutation leading to reclassification.

Study design

The unPAD study is an international multicenter observational cohort study based on the ESID online Registry data. Repeated calls for participation were published in the ESID Newsletter and on the ESID website. Furthermore, when participating centers indicated they knew of other centers who might be interested in participating, we contacted these centers. Until now, 20 centers from 10 countries actively participate in this study by collecting their data in the level 1 and level 2 forms of the ESID online Registry and have agreed to join the study (see list in the acknowledgements).

Analyses on variables at diagnosis will be conducted from 2022 onwards. The unPAD study is an ongoing study, there is still an open invitation for researchers in the field to participate in the study. The unPAD study will be running as long as the investigators expect additional information can be gained from another round of analysis, which will by nature mean a longer follow-up period than in the analyses performed before.

Variables at baseline and during follow-up

For all patients, baseline characteristics are being registered at first registration and yearly thereafter in the so-called level 1 forms. The level 1 form contains data on demographic characteristics, family history, consanguinity, IEI diagnosis, and treatment (**Table 6.3**). More detailed characteristics of the patients can be registered in level 2 forms, including detailed data on demographical, clinical and immunological characteristics, including data on additional investigations, such as lung function, gastroscopy, and Chest CT-scan (**Table 6.3**). Consecutive follow-up forms can be added indefinitely (shown in S1 Table).

Table 6.3. Overview of variables included in the unPAD study.

Variable	Definition
General (level 1)	
Patient	
Patient consent	Signed/Not applicable (only if deceased) For minors, parents or the legal guardian must give their written consent.
Date of birth	Year; Month (month only if <12 years of age)
Country of current residence	This should be the country where the patient has his permanent residence, i.e. where he/she lives for the majority of the year. If the patient stays in the current country for a longer period, but only temporarily (e.g. for specialized medical treatment or seasonal work), his/her country of origin should be selected.
Sex	Male/Female
Familial case	Defined as another patient with a diagnosed primary immunodeficiency in the genetic family (e.g. parents, siblings, grandparents).
Consanguinity of parents	Defined as genetically related parents or other ancestors (e.g. grandparents) of the patient.
Documenting Centre	Name of the center from which the data originate.
Way to Diagnosis	
Date of first clinical diagnosis of IEI	Year; Month; Day The date when this patient was first diagnosed with a primary immunodeficiency based on clinical features and laboratory values.
First IEI-related symptom(s)	· Infection · Immune dysregulation (lymphoproliferation, granuloma formation, autoimmunity, inflammatory bowel disease, celiac disease, vasculitis, eczema, autoinflammatory disease) · Malignancy · Syndrome manifestations · Other · No IEI-related symptoms at all

Table 6.3. Continued.

Variable	Definition
General (level 1)	
Date of onset of symptoms	Year; Month The year and month when the first symptoms suggestive of an IEI (see above) appeared in this patient, based on the physician's judgement.
IEI Diagnosis	
Current IEI Diagnosis	Defined as the most recent IEI diagnosis.
Affected gene	The gene in which disease-causing mutation(s) have been found in this patient.
Status	
Current status	· Alive · Deceased · Lost to follow-up · Discharged after complete recovery
Current Ig replacement	Yes/No
Did the patient ever receive immune modifying treatment?	Yes/No
Did the patient ever suffer from a malignancy?	Yes/No
HSCT	Yes/No
Splenectomy	Yes/No
Gene therapy	Yes/No
unPAD study (level 2)[a] first registration	
Clinical presentations (multiple answer)	· Recurrent ENT and airway infections · Failure to thrive from early infancy · Recurrent pyogenic infections · Unusual infections or unusually severe course of infections · Recurrent infections with the same type of pathogen · Autoimmune or chronic inflammatory disease; lymphoproliferation
Clinically most important clinical presentation (single answer)	· Recurrent ENT and airway infections · Failure to thrive from early infancy · Recurrent pyogenic infections · Unusual infections or unusually severe course of infections · Recurrent infections with the same type of pathogen · Autoimmune or chronic inflammatory disease; lymphoproliferation

Table 6.3. Continued.

Variable	Definition
General (level 1)	
Bacterial infections	Any major bacterial infection (+ which micro-organism)? · Pneumonia · Meningitis · Osteomyelitis · Liver Abscess · Other major infection
Frequently recurring infections	· Upper respiratory tract · Lower respiratory tract · Gastrointestinal tract · Urinary tract · Skin · Other
Unusual infections	· Severe viral · Opportunistic · Parasitic
Inflammatory bowel disease/ allergic manifestations	Inflammatory bowel disease is subdivided in 'biopsy-proven' and 'clinically suggestive, but not biopsy-proven'. Allergic manifestations are subdivided in 'proven with sensitization' and 'clinically suggestive, but not proven by sensitization'.
Chronic organ pathology	· Hepatomegaly · Splenomegaly (splenectomy ever performed?) · Chronic liver disease · Bronchiectasis · Parenchymal lung disease · Hearing impairment (not congenital) · Other
Autoimmunity	· Auto-immune hemolytic anemia · Auto-immune granulocytopenia · Auto-immune thrombocytopenia · Other
Malignancy and other manifestations	The type of malignancy and/or of other manifestations has to be specifically defined.
Medication	Daily immunosuppressive drugs or drugs that may cause hypogammaglobulinemia as a side effect (currently in use or stopped less than three months before the diagnosis of hypogammaglobulinemia).
Diagnostic vaccination response measurements	· Tetanus · Pneumococcal polysaccharide · Other

Table 6.3. Continued.

Variable	Definition
General (level 1)	
Virological analysis	· HCV-RNA · HIV-DNA · EBV-DNA · CMV-DNA
Instrumental data	· Lung function; FEV1 · HRCT thorax · Gastroscopy
Blood counts/ Immunoglobulins/ sensitization	· Laboratory values at time point closest to the diagnosis (leukocytes, neutrophils, lymphocytes, eosinophils, basophils, monocytes) · Laboratory values at time point closest to diagnosis before start of Ig-replacement (IgG, IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, M-protein) · Sensitization (specific IgE, skin prick test)
Lymphocyte subsets/ auto-antibodies	· Laboratory values at time point closest to diagnosis (CD3+, CD3+CD4+, CD3+CD8+, CD19+CD20+, CD3-CD16/56+, CD20+CD27+IgD-, CD19+CD38++IgM++, CD19+CD27-IgM+IgD+, CD19+CD27+IgM+IgD+, CD19+CD27+IgM+IgD-, CD19+CD27+IgM-IgD-) · Auto-antibodies (ANA, TPO-antibodies)

Table 6.3. [a] Follow-up forms (shown in S1 Table) can be added indefinitely.
Abbreviations: ANA, antinuclear antibody; CD, cluster of differentiation; CMV, cytomegalovirus; DNA, deoxyribonucleic acid; EBV, Epstein-Barr Virus; e.g., exempli gratia; ENT, ear-nose-throat; IEI, inborn error of immunity; FEV1, forced expiratory volume in 1 second; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HRCT, high-resolution computed tomography; HSCT, hematopoietic stem cell transplantation; Ig, immunoglobulin; RNA, ribonucleic acid; TPO, thyroid peroxidase; unPAD, unclassified primary antibody deficiency.

Data collection and storage

The registered patient data are stored on secure servers at the University Hospital Freiburg, Freiburg, Germany, using a study code. Data transfer is SSL encrypted. These pseudonymized data can only be traced back to the patient by the treating physician or documentation specialist of the center in question, not by the unPAD research team, following the European legal data protection provisions. Identifying data (e.g., name, place of residence) are stored on a separate server to which third parties have no access. The system structure of the ESID online database has been described by Perner et al. and Guzman et al [16,21]. Before registration of patient data is possible, a participating center must have signed a contract and obtained logins for the database system. The database is designed to be used for long-term documentation. It offers the possibility to add any number of visit dates for a given patient. Participating centers are asked to update their patients' data at least once a year. The database has an inbuilt automatic quality assurance system including field type, range and plausibility

checks (e.g., date of death must be later than date of birth). Some fields are mandatory, which means that data cannot be stored unless these fields are completed. Taking into account that the data are sometimes not known or currently not available to the documentalist, the boxes 'truly unknown' or 'currently unknown' can be checked. All patient data collected in the level 1 and level 2 forms will be fully monitored before data extraction for analysis in the unPAD study. In case of missing data or inconsistencies, the unPAD research team will contact the participating centers to resolve these issues.

Sample size

In order to be able to accurately describe unPAD patients, we aim to collect data on as many patients as possible. Based on the amount of registered unPAD patients in the ESID online Registry, we aim to include at least 1,000 patients. This number will allow analysis of the demographical, clinical, and immunological characteristics (at presentation and during follow-up) and of the risk of complications in potentially meaningful subgroups.

Statistical analysis

Statistical analyses will be performed with IBM SPSS Statistics and/or R (most recent versions). Data quality will be secured by the thorough monitoring process before data extraction. After extraction, the data will be cleaned and preprocessed supported by the standard set of descriptive statistics plus visualization techniques. The most suitable method for dealing with missing variables will be determined for each variable in collaboration between data analysts and domain experts (e.g., types of imputation, exclusion from analyses). We will use cluster analysis (with bootstrapping) plus supervised and unsupervised machine learning for subgroup classification using all variables together as well as (combinations of) subsets of demographical, clinical and immunological characteristics. In addition, we will use regression analysis and machine learning to create and evaluate models for predicting health-related outcome variables such as bronchiectasis. Appropriate evaluation metrics will be applied for these models depending on their type, such as R^2, accuracy, mean absolute error (MAE), root mean squared error ((R)MSE), and area under the receiver operating characteristic curve (ROC-AUC). A p-value <0.05 with correction for multiple testing when appropriate will be considered statistically significant, and/or a 95% confidence interval (CI) not containing 0, where applicable.

DISCUSSION

Most hypogammaglobulinemia patients, including those with CVID, still lack a definitive genetic diagnosis. The unPAD study has been designed to investigate 'unclassified antibody deficiency' and has the intention to describe in detail all types of PAD patients *without* an identified specific monogenetic disease-causing mutation regarding their demographical, clinical, and immunological characteristics at presentation and during follow-up. UnPAD patients form a highly heterogenous group and will remain so unless classification into clinically meaningful subgroups can be made. Efforts to stratify patients into different subgroups according to genetic screening, B- and T-cell studies [22–26] and clinical presentations [27] have been made for CVID patients. A larger group of patients suffers from a range of combinations of immunoglobulin deficiencies where the CVID definition is not met (referred to in the literature as idiopathic hypogammaglobulinemia [28], CVID-like disorder [29], IgG isotype deficiency [30], or unclassified hypogammaglobulinemia [31], and by us as unPAD). However, efforts to stratify patients into different subgroups have not yet been made for these patients. Because these disorders form a heterogenous and phenotypically overlapping group, correct classification is a real challenge. It is important to realize that current classifications (ESID Clinical Working Definitions, IUIS) are mainly based upon the results of immunological laboratory investigations, while it is not clear how *clinically* useful such a basis for classification really is. In addition to the current laboratory classification approach, we therefore plan a new, broader clinical classification approach. By grouping patients also based on clinical presentations and complications, we aim to subclassify unPAD patients to support identification of those patients with higher risks of complications. These patients could then be monitored for specific complications or be treated differently according to subtype. This will ultimately shed light on more personalized intervention approaches. In addition, the potential identification of more homogenous subgroups can help to unravel the genetic background of unPAD patients. This information will help to guide clinicians to answer the question: "*what should I do with this individual unPAD patient?*".

This is important. Although doctors are inclined to consider patients with hypogammaglobulinemia who do not match the CVID diagnostic criteria to be clinically mild, CVID and unPAD patients comprise phenotypically overlapping groups. On the one hand, the often milder affected 'infection-only' group of CVID patients share very similar disease courses to patients currently classified as unPAD. On the other hand, certain subgroups of unPAD patients suffer from similar immune dysregulation features as described in CVID [15]. The unPAD study can improve PAD patient care by identifying subgroups at risk for serious complications, implying different therapeutic consequences for these patients.

The unPAD study will be the largest study on unPAD patients to date. Of all centers participating in the ESID online Registry, 20 have indicated to participate in the unPAD study so far (13 pediatric and 7 adult centers). Of these, 10 centers have already been fully monitored during a site visit, resulting in 1010 patients who have been monitored at this moment. This

was done as preliminary work to find out whether we would achieve sufficient statistical power. This large set of patient data provides significant statistical power to not only describe the clinical presentation, prognosis, and treatment of unPAD in detail, but also to determine whether subgroups can be identified based on demographical, clinical, and immunological characteristics.

The unPAD study has its limitations. Due to lack of international consensus, the local diagnostical, treatment and follow-up protocols may differ between centers. For instance, not all patients will have undergone complete pulmonary examinations (e.g., spirometry and chest HRCT), which may lead to an underestimation of the frequency of bronchiectasis or interstitial lung disease. There will be variability in data entry practices: e.g. some centers will only record IgA deficiency if patients require active management and the adherence with annual data updating will be dependent on available resources. Moreover, facilities for genetic testing differ between centers. Therefore, a subgroup of patients with a nonidentified genetic diagnosis may be hidden in the clinically defined unPAD cohort who should actually be reclassified to a monogenetic IEI form.

The most important strength of the study is that *all* data will be monitored and – if necessary – corrected and supplemented. The usefulness and quality of data extracted from patient registries depends on correct data entry. It is thus of utmost importance for the data quality assurance to review and check the data of any newly added patient. Problems that can occur during registration of PAD patient data are, for example, entering incorrect numbers of immunoglobulins and lymphocyte subpopulations by typing errors, using wrong units (cells/ul instead of 10^9/l in lymphocyte subpopulations), misinterpretation of vaccine responses and incomplete clinical manifestations hidden under 'other options'. Furthermore, the ESID online Registry can only indicate whether a gastroscopy or chest HRCT-scan has been performed, and if so, whether the result was normal or abnormal, but the exact findings cannot be registered in the system. A monitor site visit provides the opportunity to also retrieve these detailed data, which can provide very valuable additional information.

The unPAD study is an ongoing study and explicitly reaches out to other researchers and clinicians in the field of PAD to join the study. This initiative aims to become a platform that facilitates future collaborative research in the field. We expect that our study will give more insight in the demographical, clinical, and immunological characteristics of unPAD patients and will identify which subgroups are at risk for infections or complications based on immune dysregulation, enabling the development of personalized follow-up and treatment plans.

REFERENCES

1. de Vries E. Patient-centred screening for primary immunodeficiency, a multi-stage diagnostic protocol designed for non-immunologists: 2011 update. Clin Exp Immunol. 2012 Jan;167(1):108–19.
2. Gathmann B, Grimbacher B, Beaute J, Dudoit Y, Mahlaoui N, Fischer A, et al. The European internet-based patient and research database for primary immunodeficiencies: results 2006-2008. Clin Exp Immunol. 2009 Sep;157 Suppl:3–11.
3. Bousfiha AA, Jeddane L, Ailal F, Al Herz W, Conley ME, Cunningham-Rundles C, et al. A phenotypic approach for IUIS PID classification and diagnosis: guidelines for clinicians at the bedside. J Clin Immunol. 2013 Aug;33(6):1078–87.
4. Picard C, Al-Herz W, Bousfiha A, Casanova J-L, Chatila T, Conley ME, et al. Primary Immunodeficiency Diseases: an Update on the Classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015. J Clin Immunol. 2015 Nov;35(8):696–726.
5. De Vries E. Patient-centred screening for primary immunodeficiency: A multi-stage diagnostic protocol designed for non-immunologists. Clin Exp Immunol. 2006;
6. Sigstad HMH, Stray-Pedersen A, Frøland SS. Coping, quality of life, and hope in adults with primary antibody deficiencies. Health Qual Life Outcomes. 2005 May;3:31.
7. Titman P, Allwood Z, Gilmour C, Malcolmson C, Duran-Persson C, Cale C, et al. Quality of life in children with primary antibody deficiency. J Clin Immunol. 2014 Oct;34(7):844–52.
8. Rider NL, Kutac C, Hajjar J, Scalchunes C, Seeborg FO, Boyle M, et al. Health-Related Quality of Life in Adult Patients with Common Variable Immunodeficiency Disorders and Impact of Treatment. J Clin Immunol. 2017 Jul;37(5):461–75.
9. Picard C, Bobby Gaspar H, Al-Herz W, Bousfiha A, Casanova J-L, Chatila T, et al. International Union of Immunological Societies: 2017 Primary Immunodeficiency Diseases Committee Report on Inborn Errors of Immunity. J Clin Immunol. 2018 Jan;38(1):96–128.
10. Bonilla FA, Barlan I, Chapel H, Costa-Carvalho BT, Cunningham-Rundles C, de la Morena MT, et al. International Consensus Document (ICON): Common Variable Immunodeficiency Disorders. J allergy Clin Immunol Pract. 2016;4(1):38–59.
11. Chapel H, Cunningham-Rundles C. Update in understanding common variable immunodeficiency disorders (CVIDs) and the management of patients with these conditions. Br J Haematol. 2009 Jun;145(6):709–27.
12. Ameratunga R, Woon S-T. Perspective: Evolving Concepts in the Diagnosis and Understanding of Common Variable Immunodeficiency Disorders (CVID). Clin Rev Allergy Immunol. 2020 Aug;59(1):109–21.
13. Bousfiha A, Jeddane L, Picard C, Al-Herz W, Ailal F, Chatila T, et al. Human Inborn Errors of Immunity: 2019 Update of the IUIS Phenotypical Classification. J Clin Immunol. 2020 Jan;40(1):66–81.
14. Seidel MG, Kindle G, Gathmann B, Quinti I, Buckland M, van Montfrans J, et al. The European Society for Immunodeficiencies (ESID) Registry Working Definitions for the Clinical Diagnosis of Inborn Errors of Immunity. J allergy Clin Immunol Pract. 2019 Jul;7(6):1763–70.
15. Janssen LMA, Bassett P, Macken T, van Esch J, Pruijt H, Knoops A, et al. Mild Hypogammaglobulinemia Can Be a Serious Condition. Front Immunol. 2018;9:2384.
16. Guzman D, Veit D, Knerr V, Kindle G, Gathmann B, Eades-Perner AM, et al. The ESID Online Database network. Bioinformatics. 2007 Mar;23(5):654–5.

17. Aghamohammadi A, Montazeri A, Abolhassani H, Saroukhani S, Pourjabbar S, Tavassoli M, et al. Health-related quality of life in primary antibody deficiency. Iran J Allergy Asthma Immunol. 2011 Mar;10(1):47–51.
18. Jorgensen GH, Gardulf A, Sigurdsson MI, Arnlaugsson S, Hammarstrom L, Ludviksson BR. Health-related quality of life (HRQL) in immunodeficient adults with selective IgA deficiency compared with age- and gender-matched controls and identification of risk factors for poor HRQL. Qual Life Res. 2014 Mar;23(2):645–58.
19. Quinti I, Di Pietro C, Martini H, Pesce AM, Lombardi F, Baumghartner M, et al. Health related quality of life in common variable immunodeficiency. Yonsei Med J. 2012 May;53(3):603–10.
20. Registry Working Party Informed Patient Consent [Internet]. Available from: https://esid.org/Working-Parties/Registry-Working-Party/Informed-Patient-Consent
21. Eades-Perner A-M, Gathmann B, Knerr V, Guzman D, Veit D, Kindle G, et al. The European internet-based patient and research database for primary immunodeficiencies: results 2004-06. Clin Exp Immunol. 2007 Feb;147(2):306–12.
22. Warnatz K, Denz A, Drager R, Braun M, Groth C, Wolff-Vorbeck G, et al. Severe deficiency of switched memory B cells (CD27(+)IgM(-)IgD(-)) in subgroups of patients with common variable immunodeficiency: a new approach to classify a heterogeneous disease. Blood. 2002 Mar;99(5):1544–51.
23. Piqueras B, Lavenu-Bombled C, Galicier L, Bergeron-van der Cruyssen F, Mouthon L, Chevret S, et al. Common variable immunodeficiency patient classification based on impaired B cell memory differentiation correlates with clinical aspects. J Clin Immunol. 2003 Sep;23(5):385–400.
24. Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, et al. The EUROclass trial: defining subgroups in common variable immunodeficiency. Blood. 2008 Jan;111(1):77–85.
25. Picat M-Q, Thiébaut R, Lifermann F, Delbrel X, Adoue D, Wittkop L, et al. T-cell activation discriminates subclasses of symptomatic primary humoral immunodeficiency diseases in adults. BMC Immunol. 2014 Mar;15:13.
26. Rösel AL, Scheibenbogen C, Schliesser U, Sollwedel A, Hoffmeister B, Hanitsch L, et al. Classification of common variable immunodeficiencies using flow cytometry and a memory B-cell functionality assay. J Allergy Clin Immunol. 2015 Jan;135(1):198–208.
27. Chapel H, Lucas M, Lee M, Bjorkander J, Webster D, Grimbacher B, et al. Common variable immunodeficiency disorders: division into distinct clinical phenotypes. Blood. 2008 Jul;112(2):277–86.
28. Driessen GJ, Dalm VASH, van Hagen PM, Grashoff HA, Hartwig NG, van Rossum AMC, et al. Common variable immunodeficiency and idiopathic primary hypogammaglobulinemia: two different conditions within the same disease spectrum. Haematologica. 2013 Oct;98(10):1617–23.
29. van de Ven AAJM, van Montfrans JM. Clinical complications in pediatric CVID are not restricted to patients with severely reduced class-switched memory B cells. Vol. 22, Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. England; 2011. p. 347–8.
30. Filion CA, Taylor-Black S, Maglione PJ, Radigan L, Cunningham-Rundles C. Differentiation of Common Variable Immunodeficiency From IgG Deficiency. J allergy Clin Immunol Pract. 2019 Apr;7(4):1277–84.
31. Kutukculer N, Gulez N. The outcome of patients with unclassified hypogammaglobulinemia in early childhood. Pediatr Allergy Immunol. 2009 Nov;20(7):693–8.
32. Janssen LMA, Macken T, Creemers MCW, Pruijt JFM, Eijk JJJ, De Vries E. Truly selective primary IgM deficiency is probably very rare.

Supplementary Table 6.1. Overview of variables included in the follow-up forms of the ESID online Registry used in the unPAD study.

Variable (since last documentation)	**Definition**
unPAD study (level 2)[a]	
Follow-up	· Visit date · Current weight and Height, BMI
Bacterial infections	Any major bacterial infection (+ which micro-organism)? · Pneumonia · Meningitis · Osteomyelitis · Liver Abscess · Other major infection
Frequently recurring infections[b]	· Upper respiratory tract · Lower respiratory tract · Gastrointestinal tract · Urinary tract · Skin · Other
Unusual infections	· Severe viral · Opportunistic · Parasitic
Inflammatory bowel disease/ allergic manifestations	Inflammatory bowel disease is subdivided in 'biopsy-proven' and 'clinically suggestive, but not biopsy-proven'. Allergic manifestations are subdivided in 'proven with sensitization' and 'clinically suggestive, but not proven by sensitization'.
Chronic organ pathology	· Hepatomegaly · Splenomegaly (splenectomy ever performed?) · Chronic liver disease · Bronchiectasis · Parenchymal lung disease · Hearing impairment (not congenital) · Other
Autoimmunity	· Auto-immune haemolytic anaemia · Auto-immune granulocytopenia · Auto-immune thrombocytopenia · Other
Malignancy and other manifestations	The type of malignancy and/or of other manifestations has to be specifically defined.
Medication	Daily immunosuppressive drugs or drugs that may cause hypogammaglobulinemia as a side effect (currently in use or stopped less than three months before this documentation).

Supplementary Table 6.1. Continued.

Variable (since last documentation)	**Definition**
Diagnostic vaccinations	· Tetanus · Pneumococcal polysaccharide · Other
Virological analysis	· HCV-RNA · HIV-DNA · EBV-DNA · CMV-DNA
Instrumental data	· Lung function; FEV1 · HRCT thorax · Gastroscopy
Blood counts/ Immunoglobulins/ sensitization	· Laboratory values at time point closest to this documentation (leukocytes, neutrophils, lymphocytes, eosinophils, basophils, monocytes) · Laboratory values at time point closest to this documentation (IgG, IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, M-protein) · IgG measured under Ig substitution (Yes/No/Unknown) · Sensitization (specific IgE, skin prick test)
Lymphocyte subsets/ auto-antibodies	· Laboratory values at time point closest this documentation (CD3+, CD3+CD4+, CD3+CD8+, CD19+CD20+, CD3-CD16/56+, CD20+CD27+IgD-, CD19+CD38++IgM++, CD19+CD27-IgM+IgD+, CD19+CD27+IgM+IgD+, CD19+CD27+IgM+IgD-, CD19+CD27+IgM-IgD-) · New results auto-antibodies (ANA, TPO-antibodies)

Supplementary Figure 6.1. [a] *Follow-up forms can be added indefinitely.*
[b] *Defined as acute respiratory infections occurring 8 episodes per year if age < 3 years and/or 6 episodes per year if age ≥ 3 years.*
Abbreviations: ANA, antinuclear antibody; CD, cluster of differentiation; CMV, cytomegalovirus; DNA, deoxyribonucleic acid; EBV, Epstein-Barr Virus; e.g., exempli gratia; ENT, ear-nose-throat; IEI, inborn error of immunity; FEV1, forced expiratory volume in 1 second; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HRCT, high-resolution computed tomography; HSCT, hematopoietic stem cell transplantation; Ig, immunoglobulin; RNA, ribonucleic acid; TPO, thyroid peroxidase; unPAD, unclassified primary antibody deficiency.

PART II

Early detection of antibody deficiency

Chapter 7

Which triggers could support timely identification of primary antibody deficiency? A qualitative study using the patient perspective

Lisanne MA Janssen, MD, Kim van den Akker, MD, Mohamed A Boussihmad, Bsc, Esther de Vries, MD, PhD

Orphanet J Rare Dis. 2021; 16(1):289

ABSTRACT

Background

Patients with predominantly (primary) antibody deficiencies (PADs) commonly develop recurrent respiratory infections which can lead to bronchiectasis, long-term morbidity and increased mortality. Recognizing symptoms and making a diagnosis is vital to enable timely treatment. Studies on disease presentation have mainly been conducted using medical files rather than direct contact with PAD patients. Our study aims to analyze how patients appraised their symptoms and which factors were involved in a decision to seek medical care.

Methods

14 PAD-patients (11 women; median 44, range 16-68yrs) were analyzed using semi-structured interviews until saturation of key emergent themes was achieved.

Results

Being always ill featured in all participant stories. Often from childhood onwards periods of illness were felt to be too numerous, too bad, too long-lasting, or antibiotics were always needed to get better. Recurrent or persistent respiratory infections were the main triggers for patients to seek care. All participants developed an extreme fatigue, described as a feeling of physical and mental exhaustion and thus an extreme burden on daily life that was not solved by taking rest. Despite this, participants tended to normalize their symptoms and carry on with usual activities. Non-immunologists, as well as patients, misattributed the presenting signs and symptoms to common, self-limiting illnesses or other 'innocent' explanations. Participants in a way understood the long diagnostic delay. They know that the disease is rare and that doctors have to cover a broad medical area. But they were more critical about the way the doctors communicate with them. They feel that doctors often don't listen very well to their patients. The participants' symptoms as well as the interpretation of these symptoms by their social environment and doctors had a major emotional impact on the participants and a negative influence on their future perspectives.

Conclusions

To timely identify PAD, 'pattern recognition' should not only focus on the medical 'red flags', but also on less differentiating symptoms, such as 'being always ill' and 'worn out' and the way patients cope with these problems. And, most important, making time to really listen to the patient remains the key.

INTRODUCTION

Rare diseases are defined as occurring in less than 1:2,000 people. However, since there are around 8,000 rare diseases, some 30 million people in both Europe and in the USA suffer from a rare disease. This is an important problem for the patients as well as for the society they live in because rare diseases are often diagnosed late, especially when they share symptoms with common diseases, leading to delayed and inadequate treatment. As a consequence, these patients suffer a decreased life quality as well as a decreased potential for societal participation (school, work) [1,2].

Predominantly (primary) antibody deficiencies (PADs) are a typical example of such difficult-to-recognize rare diseases [3]. Hypogammaglobulinemias are by far the most common forms of PAD, comprising nearly half of all primary immunodeficiency (PID) diagnoses [4–6]. Affected persons commonly develop recurrent otitis media, sinusitis, and pneumonia. Recurrent pneumonias can lead to bronchiectasis, which serves as a negative factor for long-term morbidity and mortality. Since the introduction of immunoglobulin replacement therapy, there have been dramatic improvements in survival [7,8]. Recognizing symptoms and making a diagnosis is therefore vital to enable timely treatment.

Although PADs are the most common primary immunodeficiencies (PIDs) in humans, they are still rare with a prevalence of approximately 1:25,000 to 1:110,000, depending on the type of PAD [3]. These patients often go unrecognized, because the general public as well as most healthcare professionals, who are not specialized in immunodeficiency, do not consider PAD in patients with recurrent "normal" infections. Because of the variability of presenting clinical manifestations, patients visit various physicians of different specialties in search of a diagnosis, which increases the risk of missing the overarching clinical pattern and thereby overlooking the underlying hypogammaglobulinemia [9]. Timely diagnosis and treatment will likely result in improved clinical and quality-of-life outcomes for patients with PAD, higher participation in society (school, work) and lower healthcare costs [10–15]. Reducing diagnostic delay is therefore crucial.

Studies on disease presentation have mainly been conducted using medical files rather than direct contact with PAD patients. We aimed to explore the presenting pattern of PAD from the perspective of patients and to identify factors that affect a correct and timely diagnosis, by exploring the period leading to the PAD diagnosis through narrative patient interviews.

MATERIALS AND METHOD

Design and setting

Patient experiences regarding their journey to receiving a diagnosis and adequate clinical care are best understood via an in-depth qualitative approach. Through individual semi-structured interviews with patients who had a diagnosis of PAD, new insights derived from their perspective were sought. Their experiences and reasoning regarding complaints and the diagnostic delay they suffered were explored. New interviews were conducted until saturation of key emergent themes was achieved, meaning additional interviews were no longer adding new themes to the data set. Methods and results are reported according to the COREQ checklist [16].

Population

Participants were recruited by an email sent by the Dutch patient organization for primary immunodeficiency diseases to all their members ('Stichting voor Afweerstoornissen', SAS). The email invited the members to participate in an interview of about one hour at a place of their choice and stated that it would be audiotaped. All participants provided written and audio informed consent. The interviews were primarily with study participants but the contribution of a relative, if present, was also welcomed.

Data collection

The interviews were performed in October and November 2016 at the patients' homes. The interviews were conducted by a master student in the last year of medical education (KvA). Questions were semi-structured, designed to address those items which the interviewer wished to raise, and also allowing participants the freedom to express their own perspective and to offer an opportunity for serendipitous findings. The questions (overview in **Supplementary Table 7.1**) were based on the literature related to clinical characteristics of primary antibody deficiency [9,17–19] and to psychosocial theories relating to symptom appraisal and care-seeking [20]. All interviews were reviewed and discussed with an experienced medical immunologist with expertise in qualitative methods (EdV).

Data analysis

All interviews were audiotaped and literally transcribed (KvA and MAB), and analysed using the framework method [21]. Data were anonymized by removing any information that could identify the patient. Transcripts were read and re-read to ensure familiarization, and independently coded (MAB and LJ). The coding was reviewed by a third coder (EdV), to ensure that the type and range of codes applied was appropriate and consistent. The coding lists were used to develop a framework organized into categories. In total, we identified 154 codes we divided into 11 categories, which were organized in 3 themes (**Supplementary**

Table 7.2). The coding was finalized using the software package Atlas.ti Version 7.1.5 (Berlin, Germany), and the coded data were exported (MAB). This export was read, re-read and then summarized for each of the 14 participants of the study. Each category was then interpreted using an analytical memo to explore emerging themes and concepts.

RESULTS

In total, 14 participants were interviewed. Interviews lasted from 45 to 105 minutes. Eleven women and three men participated, median age 44 years, range 16-68; all participants were Dutch. Participant characteristics are summarized in **Table 7.1**. Three interviewees were accompanied by a relative during the interview. The results are presented under subheadings reflecting the main steps in the diagnostic pathway, namely: presentation of PAD and participants' interpretation of symptoms, progression of symptoms and realization that something is really wrong, starting the patient journey, doctors' interpretation of symptoms, and triggers to diagnosis. Participants were also asked to reflect on care provision and on the emotional toll of the diagnostic process.

Presentation of primary antibody deficiency and participants' interpretation of symptoms

The presenting features of PAD described by participants were diverse, intermittent and sometimes non-specific, covering a broad range of behavioural and physical changes (**Table 7.1**). Being always ill featured in all participant stories. It often occurred from childhood onwards and was considered to be a problem by participants and/or their parents when periods of illness were felt to be too numerous, too bad, too long-lasting, or when antibiotics were always needed to get better.

> *Then I got my penicillin course, then it was over within two days, but then the penicillin course was over again and two days later it started all over again. (Participant 4)*

Many participants thought recurrent infections to be normal for children, or in some cases due to atopic disease. Seven adult participants just felt ill and did not have a particular explanation for why they were more often ill than others but assumed that the symptoms would probably resolve with time. Participants tended to downplay and/or normalize their symptoms.

> *When it started with asthma, I had two to four respiratory tract infections a year. That's not very strange, it is not what strikes you as abnormal if you have asthma. (Participant 9)*

> *I think everybody coughs sometimes with a little mucus, but then I don't feel very sick. Usually, it resolves with time. Eventually, when I was diagnosed with CVID, they did a Chest-CT. Then they saw the beginning damage that fits the clinical picture and lungs. They also said: 'how is that possible, you said you only had pneumonia once, but from looking at the scan it seems as if it really cannot have been just once'. (Participant 10)*

Fatigue was present both before and after the diagnosis in many participants and was described as a feeling of physical and mental exhaustion and thus an extreme burden on daily life. Most participants recalled that they slept very well, but still remained tired. The decrease in energy level could result in the need for an afternoon nap.

> *And really a lot of fatigue, I slept for three hours during the day and ten hours at night. I really slept thirteen to fifteen hours a day. Just to keep up. (Participant 1)*

> *I spoke about it with my sister recently. I always thought, everybody works the whole day, five days a week, but I actually can't do that. It would tire me so badly, it's not possible. (Participant 6)*

Participants described they thought they exerted themselves too much, causing their symptoms themselves by working too hard, taking care of their whole family or by being too active socially. The degree of fatigue could be significant before the participant really labelled it as a problem. Eleven participants reported their fatigue was so severe it took away all their free time, because after a work/school day there was no energy left for social activities.

> *Then I sleep the whole night, being just able to fulfil the expectations set during the day. I don't have any free time left, because when I come home, I go to sleep, and then another day begins. (Participant 10)*

Participants had comorbidities, complications or misdiagnoses, and tended to attribute their symptoms to these conditions. For example, a participant with iron deficiency anaemia attributed her fatigue solely to this condition, and a participant with Graves' disease attributed her fatigue solely to that. Whereas in both participants these conditions could well be complications of their – unrecognized – PAD. Another participant with a misdiagnosis of asthma thought her fatigue was the result of needing more potent inhalers. In total, four participants (1, 7, 9 and 12) attributed their symptoms to asthma.

> *At that time I got diagnosed with asthma, for which I had to use inhalers that just did not work. (Participant 7)*

Participants carried on with their usual activities despite significant limiting symptoms.

> *Just tired... I always went to work anyway, ...what good is it to you to lie down on the couch all day. (Participant 4)*

Table 7.1. Symptom attribution and delay before diagnosis.

Patient	Age (years)			Delay (years)
	at start of symptoms	at time of diagnosis	at time of interview	
1, F, CVID	28	33	38	5
2, F, sIgAdef	13	32	35	19
3, F, CVID	26	29	35	3
4, F, CVID	43	46	51	3
5, M, agammaglobulinemia	4	13	59	9
6, F, CVID	45	51	57	6
7, F, unPAD	0	5	36	5
8, M, CVID	5	40	58	35
9, F, unPAD	22	42	46	20
10, F, CVID	8	23	24	16
11, M, sIgAdef	0	4	16	4
12, F, IgG subclass deficiency	1	40	44	39
13, F, Good syndrome	not precisely known	68	68	>30
14, F, CVID	40	50	63	10

Table 7.1. Abbreviations: CVID, common variable immunodeficiency disorders; F, female; IgGscdef, IgG subclass deficiency; ITP, idiopathic thrombocytopenic purpura; M, male; n/a, not applicable; PID, primary

	Signs and symptoms	Patient's attribution
	Recurrent sinusitis/ otitis/ rhinitis/ pneumonia, fatigue, weight loss, anosmia, splenomegaly	Increased susceptibility due to pregnancy, being too busy and taking too little rest
	Chronic rhinitis, hypothyroidism, fatigue, stomach and bowel complaints	No considerations, but fear about the diagnosis
	Chronic cough, recurrent otitis/ bronchitis, ITP, alopecia areata, chronic fatigue	Some kind of autoimmune disorder, sensitive lungs
	Being always ill, almost continuously fever, recurrent rhinitis/ otitis/ pneumonia/ sinusitis, anosmia, fatigue, recurrent ITP, chronic diarrhoea, meningitis, inguinal lymphadenopathy, weight loss	n/a
	Recurrent meningitis/ pneumonia/ otitis/ sinusitis	XLA (after diagnosed was discovered in his brother)
	Recurrent respiratory infections/ sinusitis/ pneumonia, chronic cough, aphthous lesions, salpingitis, arthralgia, bronchial hyperreactivity, fatigue, exercise intolerance	Some kind of immune disorder
	Recurrent otitis/ rhinitis/ sinusitis, chronic cough, skin abscess, pneumonia, failure to thrive	n/a
	Recurrent otitis/ rhinitis/ sinusitis/ pneumonia/ varicella zoster/ Giardia lamblia, fatigue, warts, meningitis, anosmia	n/a
	Recurrent otitis/ sinusitis/ pneumonia/ skin infections, mumps, chickenpox (2x), asthma, Graves' disease	Initially Graves' disease and asthma, later after searching the internet an immune disorder
	Erythema nodosum, splenomegaly, enlarged supraclavicular lymph node, fatigue, oral aphthous lesions, being always ill, recurrent otitis/ sinusitis	Iron deficiency anaemia, some kind of viral infection
	Recurrent rhinitis/ otitis/ pharyngitis, fatigue, growth retardation, chronic diarrhoea	n/a
	Recurrent sinusitis/ pharyngitis/ respiratory tract infections, fatigue, multiple allergies, asthma, retropharyngeal abscess	Combination of (severe) asthma and allergies
	Iron deficiency anaemia, recurrent lymphadenopathy/ cystitis/ sinusitis/ otitis/ respiratory tract infections, fatigue, chronic diarrhoea, diverticulitis	Combination of iron deficiency anaemia, asthma and diverticulitis
	Recurrent sinusitis and pneumonia, odontogenic infections, sepsis, severe wound infection, fatigue, exercise intolerance, chronic slightly elevated body temperature	Initially viral infections in combination with psychological factors (divorce) and menopause, later after searching the internet an immune disorder

immunodeficiency; slgAdef, selective IgA deficiency; unPAD, unclassified primary antibody deficiency; XLA, X-linked agammaglobulinemia.

I have always done fitness at a fairly high level and I did that three times a week. At one point it became less and less due to fatigue. That I just couldn't manage to exercise for an hour at eight o'clock in the evening. Sometimes I had to force myself to do it, but then I just took an extra puff, so I could do it again. (Participant 1)

Often, unusual and alarming signs for PID were not recognized as unusual medical conditions by the consulting doctors, for example: ITP, alopecia areata and recurrent infections in participant 3; recurrent chicken pox in participants 8 and 9; excessive oral aphthous lesions in participant 10; recurrent meningitis in participant 5; recurrent otitis in adult patients repeatedly needing tympanostomy tubes in participants 1, 2 and 8; excessive weight loss in participants 1 and 4; salpingitis after swimming in participant 6, and impaired wound healing in participant 9.

Progression of symptoms and realization that something is really wrong

Typically, the symptomatology evolved over weeks to months, with non-specific early features such as recurrent "normal" infections, fever, chronic cough and fatigue, mimicking those of common, self-limiting illnesses. Some participants described a triggering event (thyroid disease, pregnancy, weight loss or severe wound infection after caesarean section) as starting point for a sudden increase in infections. Symptoms often progressed over time. Infections slowly became abnormally recurrent, severe and/or persistent.

It starts with only periods of coughing and then at one point it's actually all the time. (Participant 3)

Four participants (4, 6, 8 and 12) suffered from chronic rhinosinusitis and underwent sinus surgery. This resulted in only a short relief of complaints. In the sons of participant 8, multiple sinus surgeries were performed in addition to weekly nasal irrigation and polypectomy. The ENT specialist was alarmed by their voluminous medical file.

But the ENT-specialist told us: 'you are right, at the whole ENT-department we have nobody with a file as large as those of your sons'. We came there for only three years. He said: 'those files are now already bigger than the files of a fifty-year old'. (Participant 8)

Nine participants (1, 3-6, 8, 9, 12 and 14) recalled their infections only resolved with antibiotic treatment.

As soon as I got my antibiotics intravenously in the hospital, I recovered. So everyone was like, you're fine again, you can go home. So yeah, it was okay for a while, only after four episodes of six days, so four weeks of illness, they wanted to conduct further research. (Participant 1)

Participants often recalled a pattern in the complaints. In participants 1 and 12 the upper respiratory tract infection (otitis, pharyngitis and/or sinusitis) always progressed to a lower respiratory tract infection.

> *It progressed from an infection of the sinuses, to the ears and then to the throat and airways. (Participant 1)*

> *It often began with infections: lungs, sinus, yes very often my sinuses, and then it spread to my lungs and throat at the same time. (Participant 12)*

Four participants (1, 2, 3 and 8) repeatedly developed otitis – often after swimming – in adulthood and were treated with tympanostomy tubes.

> *Well, I mean, everybody might have an ear infection once a while, but every time I had it, it lasted a month. That I really had so much pain for a month, that you just wanted to hit your head against the wall, because you don't know what to do about the pain anymore. Then the doctor told me: 'yes, but antibiotics do not help against an ear infection, so I do not really want to give that'. Then it lasted just really long every time, but the GP did not think: 'oh, that is weird'. (Participant 3)*

Participants 7 and 9 realized they differed from other people when comparing the duration of recovery.

> *I often had a cold. Another person had it for two days and if I had it was for two months. (Participant 7)*

> *I played handball and that is not a 'sweet' sport, to be fair, my wounds recovered badly... That was very weird, the wound always got infected or it took four weeks to heal. With the other kids in my environment the wounds always recovered within a week or two. For me never, it always took longer. (Participant 9)*

Participant 6 recalled her infections to start rapidly and become severe in a short time.

> *It could be that one moment I thought: 'I'm going to make it', but then an hour later I would be so ill that I didn't make it. (Participant 6)*

The burden of infections was perceived to be susceptible to change. Participants pointed out that they experienced positive as well as negative fluctuations in the burden of infections due to weather conditions: four patients reported being sick throughout the year (1, 2, 4, 8); three patients were almost never ill during the summer (5, 6, 9).

All except two participants were working age and initially thought that their symptoms were a normal part of their busy lifestyle and job, but once recognized as abnormal by others (employers, colleagues) they sought help. Family members often witnessed participants struggling with symptoms and encouraged them to seek help. Five participants (1, 2, 4, 5 and 7) often recalled that their social environment thought they were sicker than they admitted themselves.

> *I just went to my job, I have often been sent home by my boss. (Participant 1)*

> *That's also what my colleagues said, how often I was at the office with a sinusitis or otitis, that everybody was like: 'you shouldn't do that, you're ill'. Yes, I am very often ill, this isn't even that bad. (Participant 1)*

Most participants were quick to seek medical advice from their GP as soon as they realized something was really wrong. However, it took most participants a long time to realize this; they continued to hope that the symptoms would simply disappear.

Starting the patient journey

The factors which triggered the seeking of care were various. One patient sought help because of the psychological stress she suffered due to the many unexplained symptoms. Recurrent or persistent infectious episodes like recurrent otitis, sinusitis, or pneumonia or endless coughing, were the main triggers for patients to seek care. Often the fatigue and strain of coping in life while hindered by all the symptoms were the reason to go – again – to the GP. Combinations of problems could also be the final push.

Participants reported cumulative barriers that led to delays to seek help. Their interpretation of the initial signs and symptoms of the disease influenced whether they sought help. Participants either got used to their symptoms or hoped their symptoms would pass by.

> *At a certain point you raise the bar and think by yourself: 'I am not using medicines or visit the doctor again'. You think: 'I just keep taking pills'. Then you raise the bar again and wait and see for another day. (Participant 1)*

> *Well, in the beginning you go to the doctor... But when you are always ill, you just don't go to the doctor anymore. (Participant 3)*

Others no longer sought help because the healthcare professional only treated symptoms instead of searching for the cause. Before the CVID diagnosis was made in patient 8, he underwent recurrent sinus surgeries resulting in only short relief of the (chronic) sinusitis. These experiences kept him from seeking help after a while. Another theme that emerged was feeling delegitimized.

This led them to feel distressed by the way they were treated, by not being believed or listened to and not being able to cope with symptoms. Participants reported the feeling that both healthcare professionals and others did not see them as having a legitimate illness and that the credibility of their symptoms was frequently questioned. Patient 3 reported that her symptoms were for a long time attributed to an unacknowledged mental health condition and which made her feel that the symptoms were not due to an underlying pathology.

> *I went to the doctor, who thought: 'I think it's not that bad how often you are sick'. So then she said: 'I think it is how you experience it, that it is in your head, but not real'. So then she initially referred me to a psychologist. (Participant 3)*

Over time, having healthcare professionals questioning their credibility made participants question the legitimacy of their symptoms too. Participants described feeling guilty for wasting healthcare professionals' time, or downgrading their symptoms by normalization, waiting till another infectious episode passed by.

Once patients began to seek a diagnosis, delays also occurred within the healthcare system. Clinicians were not often familiar with PID and were challenged by the complexity and rarity of the disease. This impacted their ability to make a differential diagnosis. Sometimes healthcare professionals seemed to have difficulties in abandoning an initial diagnosis. Participant 6 was treated for bacterial pneumonia, but her general state of health worsened with loss of condition, leading to the inability to climb the stairs. She suggested herself to screen for possible PID because of recurrent Hemophilus Influenzae pneumonia despite adequate antibiotic treatment, after which CVID was discovered. In addition to physician inflexibility, this case reveals the importance of communication concerning symptoms from everyday life as well as medical symptoms. Six participants (1, 6, 8, 9, 10 and 14) recalled their social environment forming a barrier in seeking help. The social environment of these participants downplayed the participants' complaints. They attributed the symptoms to stress/a busy life or told the participants it would resolve with time.

> *'It is probably because of the stress', people say that a lot too. (Participant 10)*

Doctors' interpretation of symptoms

Many participants recalled initially being offered an incorrect explanation for their symptoms (**Table 7.2**). Most doctors initially attributed the participant's symptoms to minor, viral illnesses, asthma, anatomical ENT-problems or to other 'innocent' explanations such as ascribing joint pains to sporting activities, episodic dyspnoea to stress-induced hyperventilation, feeling worn out to the combination of working too hard and taking care of a newborn child, erythema nodosum to mosquito bumps and exercise intolerance to menopause. In one participant XLA, despite a positive family history, was only discovered years after he already had several episodes of meningitis. In one participant, her symptoms were put down to being pregnant.

Participants were referred to several different specialists (**Table 7.2**), often only after strongly insisting on it. In two participants, their symptom attribution to psychological factors, led to multiple visits to a psychiatrist.

> *I did not have severe infections, but I couldn't do anything anymore. Well, what happens then: 'psychic, menopause, divorce'. I started believing that after a while. Then I went to a psychiatrist. I went there for years. (Participant 14)*

Two participants (2 and 8) appeared to have one or more decreased immunoglobulin isotypes years before the final diagnosis, but this was not noticed by the doctor or the doctor did not know what this meant and ignored the results. This reflects the problem that most non-immunologists have minimal or no knowledge of PID. Even treatment failure – implying an unusual disease course – did not alarm an ENT specialist to think of potential PID.

> *I had meningitis in 2011 and then I recovered and they thought it went better, but then I became sick again. Then I went to the ENT-doctor... They cleaned my sinuses. That was February 2012. Then I became sick again, they didn't understand it anymore. Then I ended up in the hospital again in April. (Participant 4)*

Triggers to diagnosis

In none of the participants, the symptoms were attributed to potential PID by the GP, except for one participant, in whom his children were already diagnosed with PID. Only two participants were referred directly to an immunologist, where referral immediately led to a correct diagnosis. Two participants were diagnosed through a positive family history, although one of them had already suffered a meningitis eight times (which had not triggered the potential diagnosis). In two participants (2 and 11) PID was incidentally discovered while screening for celiac disease (low serum IgA).

Multidisciplinary consultations can support the diagnostic process. In participant 10, who had splenomegaly, erythema nodosum and pancytopenia, one of the specialists recognized the symptom pattern and suggested to test for immunoglobulins.

> *Eventually the internist told me: 'I actually don't know what you have, I think it's sarcoidosis, but your blood doesn't show that'... 'I'm going to discuss you one more time in a multidisciplinary consultation, if we don't find it then, then we really don't know'. In that consultation I think one smartass said: 'test for antibodies'. (Participant 10)*

Alarm symptoms can trigger the diagnosis. For example, healthcare professionals were triggered to conduct additional investigations when participant 1 and 4 suffered from excessive weight loss. Sometimes PID was diagnosed while searching for another

diagnosis. Participant 1 suffered from weight loss, night sweats and splenomegaly and was screened for leukaemia or lymphoma. Instead, she was found to have CVID. Participant 3 suffered from idiopathic thrombocytopenia and alopecia and was screened for some form of autoimmune disease. Her IgG was found to be decreased instead of elevated; she was diagnosed with CVID.

> *They started searching for an autoimmune disease and they determined the total serum IgG level. They expected that to be super high, because they thought of lupus or something like that. But it was very low at that time. (Participant 3)*

Abnormal symptom patterns can trigger the healthcare professional to conduct further investigations. In participant 9 the ENT specialist noticed inflammation on the inside of the nose, usually indicating allergic rhinitis. However, allergy tests were negative and antihistamines, nasal corticosteroids and turbinate reduction did not alleviate her symptoms. This triggered the ENT specialist to refer to an immunologist.

> *When I entered, he inspected my nose and said: 'this is what an allergic nose looks like'. I said to him: 'you can say that, but nobody can prove that I have allergies'. Then they cut it out and cleaned it, but I kept having a lot of complaints. Then he started to look further. (participant 9)*

The paediatric PID patients had a shorter diagnostic delay than the adult PID patients in our study. Participant 7 suffered from recurrent otitis, rhinitis and sinusitis, as well as chronic cough, skin abscesses, pneumonia and failure to thrive since birth and was diagnosed with hypogammaglobulinemia at the age of 5 (during follow-up a diagnosis of unclassified primary antibody deficiency was made). The sons of participant 8 were diagnosed with CVID by their paediatrician, triggered by recurrent need for tympanoplasty and sinus surgery. While their father had severe and recurrent infections for several years, he was only diagnosed with CVID after this was discovered in his two children.

Participant 9 and 14 played a direct role in obtaining their PID diagnosis by searching for a cause of their symptoms on the internet and diagnosing a potential PID by themselves.

Participants in a way understand the long diagnostic delay. They know the disease is rare and a GP has to cover a broad medical area. They realize the diagnostic delay is due to not knowing, instead of not wanting to know.

> *It is of course so rare. There are so many things that doctors should be aware of that I understand that it has not been directly diagnosed. (Patient 1)*

> *I don't blame her, because you can't know everything, but I thought: 'Gosh, I felt really miserable then'. (Patient 2)*

But participants are more critical about the way the doctors communicate with them. They feel that doctors often don't listen very well to their patients. They want the doctor to acknowledge they don't know what is the matter and to refer the participant to a specialist, and to observe existing guidelines.

> *The stupid thing is that there is a pulmonology guideline for recurrent airway infections. According to the protocol he had to make a referral. He didn't know that but then at least be honest enough to say that you don't know. (Participant 9)*

Emotional toll of the diagnostic delay

During the interviews it became increasingly clear that the participants' symptoms as well as the interpretation of these symptoms by their social environment and doctors had a major emotional impact. Finally knowing they have probably had PAD for years without being diagnosed also negatively impacted their lives. Participants recalled feeling a lack of understanding by their social environment, who often questioned or downgraded their complaints.

> *Well I didn't think it was hard to accept the fatigue and just go to bed. It was often more the incomprehension of others, like: 'Gosh, already? We just started'. (Participant 1)*

> *Then they think: 'there she comes again, she is sick again'. (Participant 4)*

Eight participants stated that their symptoms had a negative influence on their future perspectives. Struggling with their untreated symptoms they made different choices in their career paths or were hampered in getting promoted.

> *It always got in the way of everything. Because he was really good at his job, he would be promoted, but then he was ill again. So another person got the job. That was always very depressing. (Wife of participant 8)*

In others, symptoms prevented them from doing what made them happy, because they felt themselves to be a burden for their social environment.

> *Concerts, yes I love that. I didn't do that in a long time, because it is the worst annoyance of every orchestra member when somebody is coughing in the hall. (Participant 6)*

> *I haven't, for example, been on vacation for years. I very often felt that I was a burden for other people. When we went on vacation and I was sick again, that's not what you want. (Participant 6)*

Many participants reported that fatigue negatively influenced multiple aspects of their social network. They often could not participate in social activities due to fatigue or had to deal with the consequences of taking part in social activities by sleeping all next day. This led to social isolation and feelings of loneliness.

> *Every time they say: 'we can't come'. At seven o'clock they lie in bed and their friends go out. So then you won't be asked anymore. (Mother of the sons of participant 8, who also have CVID)*

> *You always have to disappoint people because you have to drop out last-minute. That's why a lot of CVID patients get isolated, because at some point, you don't want to disappoint people anymore. (participant 8)*

The daily limitations due to the PAD-related complaints were a heavy mental burden for many participants. Eight patients mentioned that they had, to some degree, lost the joy in life.

> *Of course when being younger I really felt alone, sometimes even depressed. (Participant 12)*

Table 7.2: The journey towards a diagnosis of primary antibody deficiency.

Patient	The diagnostic pathway			
1	Doctor	GP	ENT specialist (1st trajectory)	ENT specialist (2nd trajectory)
	Signs and symptoms	Recurrent upper airway infections	Recurrent upper airway infections	Recurrent upper airway infections
	Attribution	n/a	Nasal polyps	n/a
	Action	Referral to ENT specialist	Polypectomy	Prednisone, antibiotics, tympanoplasty
2	Doctor	GP (1st trajectory)	ENT specialist	GP (2nd trajectory)
	Signs and symptoms	Chronic rhinitis, chronic fatigue, hypothyroidism	Chronic rhinitis	Stomach and bowel complaints, chronic fatigue, frequent GP visits
	Attribution	Chronic rhinitis not further specified	Nasal septum deviation	Gastritis not further specified
	Action	Referral to ENT specialist	Septoplasty, steroid nasal spray	Antacids, and after persistent symptoms, referral to gastro-enterologist
3	Doctor	GP (1st trajectory)	Psychologist	GP (2nd trajectory)
	Signs and symptoms	Chronic cough, recurrent otitis, burn-out symptoms	Feeling worn out, burn-out symptoms	Chronic cough, recurrent otitis, burn-out symptoms
	Attribution	Recurrent bronchitis in combination with psychological factors	The combination of being always ill, working and taking care of a newborn child	N/a
	Action	Antibiotic treatment, bronchodilators, referral to psychologist	Referral back to GP	Advise to the patient to google to find out the cause of complaints
4	Doctor	GP (1st trajectory)	Pulmonologist	ENT specialist
	Signs and symptoms	Recurrent rhinitis/ pneumonia/ sinusitis	Recurrent rhinitis/ pneumonia/ sinusitis	Recurrent rhinitis/ pneumonia/ sinusitis

Pulmonologist	Oncologist	
Chronic cough, episodic dyspnea, especially at night	Recurrent upper airway infections, weight loss, frequent hospital admission for respiratory infections, night sweats, splenomegaly	
Asthma	Leukemia, non-Hodgkin lymphoma	
Pulmonary function test, increasing the dose of inhalation corticosteroids, prophylactic antibiotics	Hospital admission, extensive examinations leading to CVID diagnosis	
Gastro-enterologist	Immunologist	
Stomach and bowel complaints, infiltrative enterocyte lesions (Marsh 1)	...	
Irritable bowel syndrome	...	
Gluten-free diet was considered, peppermint oil, referral to immunologist after IgA-deficiency was discovered	...	
Patient	Immunologist	
Chronic cough, recurrent otitis, burn-out symptoms, ITP, alopecia areata	See under 'patient'	
Some kind of auto-immune disease	Immunologic or auto-immune disorder	
Arranging own referral to immunologist/ rheumatologist	Extensive laboratory investigations after which the CVID diagnosis was made	
GP (2nd trajectory)	Pulmonologist	Immunologist
Persistent, recurrent respiratory infections, meningitis	Persistent, recurrent respiratory infections, meningitis	Persistent, recurrent respiratory infections, meningitis, inguinal lymphadenopathy, weight loss

Table 7.2: Continued.

Patient	The diagnostic pathway			
	Attribution	n/a	Obstruction of sinus drainage, bacterial pneumonia	Obstruction of sinus drainage
	Action	Referral to pulmonologist	Chest X-ray, therapeutic and prophylactic antibiotic treatment, referral to ENT specialist	Endoscopic sinus surgery
5	Doctor	GP	Immunologist	
	Signs and symptoms	Recurrent meningitis, otitis, chronic sinusitis, positive family history	Recurrent meningitis, otitis, chronic sinusitis, positive family history	
	Attribution		PID	
	Action	Referral to immunologist	Extensive laboratory investigations after which the XLA diagnosis was made	
6	Doctor	GP	Pulmonologist (1st trajectory)	Pulmonologist (2nd trajectory)
	Signs and symptoms	Recurrent respiratory infections / sinusitis / pneumonia, bronchial hyperreactivity, fatigue, exercise intolerance	Recurrent respiratory infections / sinusitis / pneumonia, bronchial hyperreactivity, fatigue, exercise intolerance	*Streptococcoccus pneumoniae* pneumonia and persistent *Haemophilus influenzae* colonization despite antibiotic treatment
	Attribution	n/a	Bacterial pneumonia and asthma	Possible CVID
	Action	Referral to pulmonologist	Sputum cultures, therapeutic and prophylactic antibiotic treatment	After discovery of low serum immunoglobulins, treatment with intravenous immunoglobulins
7	Doctor	Pediatrician		

	n/a	Bacterial pneumonia	PID
	Referral to pulmonologist	Chest X-ray, antibiotics, referral to immunologist after IgA-deficiency was discovered	Extensive laboratory investigations after which the CVID diagnosis was made

Table 7.2: Continued.

Patient	The diagnostic pathway			
	Signs and symptoms	Recurrent otitis / rhinitis / sinusitis, chronic cough, skin abscess, pneumonia, failure to thrive		
	Attribution	PID		
	Action	Extensive laboratory investigations after which the unPAD diagnosis was made		
8	Doctor	GP (1st trajectory)	Pulmonologist	ENT specialist
	Signs and symptoms	Recurrent otitis/ rhinitis/ sinusitis/ pneumonia	Recurrent otitis/ rhinitis/ sinusitis/ pneumonia	Recurrent otitis/ rhinitis/ sinusitis/ pneumonia
	Attribution	n/a	Bacterial pneumonia	Nasal septum deviation/ polyps
	Action	Referral to ENT specialist and pulmonologist	Prophylactic and repeated therapeutic antibiotic treatment	Prophylactic and repeated therapeutic antibiotic treatment
9	Doctor	GP (1st trajectory)	GP (2nd trajectory)	GP (3rd trajectory)
	Signs and symptoms	Recurrent otitis/ sinusitis/ skin infections, poor wound healing, chicken pox (2x), mumps	Dyspnea, wheezing, chronic cough	Fatigue, stomach and bowel complaints
	Attribution	Recurrent infections in infancy	Asthma	Graves' disease
	Action	None	Inhalation corticosteroids, referral to pulmonologist	Antithyroid medication
10	Doctor	GP (1st trajectory)	GP (2nd trajectory)	GP (3rd trajectory)
	Signs and symptoms	Fatigue, aphthous lesions	Erythema nodosum	Erythema nodosum + splenomegaly
	Attribution	Iron deficiency anemia	Mosquito bites	Some kind of viral infection

GP (2nd trajectory)	Immunologist	
His two sons were diagnosed with CVID by a pediatrician	Recurrent otitis/ rhinitis/ sinusitis/ pneumonia, two sons were diagnosed with CVID by a pediatrician, recurrent varicella zoster and Giardia lamblia infections, warts, anosmia	
Possible CVID	Possible CVID	
Referal to immunologist	Extensive laboratory investigations after which the CVID diagnosis was made	
Pulmonologist	ENT specialist	Immunologist
Dyspnea, wheezing, chronic cough, recurrent respiratory infections	Recurrent sinusitis and pneumonia despite PnPS and Hib vaccination and antibiotic treatment	Recurrent sinusitis and pneumonia despite PnPS and Hib vaccination and antibiotic treatment
Asthma	Possible PID	Possible PID
Increasing the dose of inhalation corticosteroids, repeatedly oral prednisolone and antibiotic treatment	Functional endoscopic sinus surgery and referral to immunologist	Extensive laboratory investigations after which the unPAD diagnosis was made
GP (4th trajectory)	Internist (1st trajectory)	Internist (2nd trajectory)
Erythema nodosum + splenomegaly, enlarged supraclavicular lymph node	Erythema nodosum + splenomegaly, enlarged supraclavicular lymph node	Erythema nodosum + splenomegaly, enlarged supraclavicular lymph node
Possible malignancy	Sarcoidosis	

Table 7.2: Continued.

Patient	The diagnostic pathway			
	Action	Iron supplementation	'Wait and see'	Blood test showed mild pancytopenia; initially 'wait and see'
11	Doctor	GP	Pediatrician (1st trajectory)	ENT specialist
	Signs and symptoms	Recurrent rhinitis/ otitis/ sinusitis, fatigue, growth retardation, chronic diarrhea	Recurrent rhinitis/ otitis/ sinusitis, fatigue, growth retardation, chronic diarrhea	Recurrent rhinitis/ otitis/ sinusitis, fatigue, growth retardation, chronic diarrhea
	Attribution		Possible celiac disease, recurrent infections in infancy	Reactive mucosa, recurrent infections in infancy
	Action	Referral to ENT specialist en pediatrician	Referral to dietician, prophylactic antibiotics after low IgA was discovered during screening for celiac disease	Tonsillectomy, adenotomy, tympanoplasty, functional endoscopic sinus surgery
12	Doctor	Pediatrician	Pulmonologist (1st trajectory)	ENT specialist
	Signs and symptoms	Recurrent sinusitis/ pharyngitis/ respiratory tract infections, fatigue	Multiple hospital admisions due to asthma (>40x)	Recurrent sinusitis/ pharyngitis/ respiratory tract infections, fatigue, retropharyngeal abcess
	Attribution	(Severe) asthma and multiple allergies	(Severe) asthma and multiple allergies	Reactive mucosa, bacterial infections
	Action	Inhalation corticosteroids, referal to pulmonologist and ENT specialist	Frequently oral prednisolone, increasing the dose of inhalation corticosteroids, repeatedly antibiotics, subcutaneous epinephrine always available	Abscess drainage, tonsillectomy, multiple sinus surgeries
13	Doctor	GP (1st trajectory)	GP (2nd trajectory)	GP (3rd trajectory)

Referral to internist	Exclusion of lymphoma after histological examination of lymph node, chest X-ray, discussion in a multidisciplinary team	After suggestion of a colleague to test for immunoglobulins, the diagnosis of CVID was made
Pediatrician (2nd trajectory)	Pulmonologist	Immunologist
Persistent infections despite prophylactic antibiotics and multipe ENT surgeries, extreme fatigue, growth retardation	Persistent infections despite prophylactic antibiotics and multipe ENT surgeries, extreme fatigue, growth retardation	Persistent infections despite prophylactic antibiotics and multipe ENT surgeries, extreme fatigue, growth retardation
Combination of recurrent infections in infancy and psychological factors	Possible CF/PCD	Possible selective IgA-deficiency
Referal to psychologist en pulmonologist	Analyses for CF and PCD were negative; referral to immunologist	Selective IgA-deficiency confirmed
Pulmonologist (2nd trajectory)		
Still frequent asthma exacerbations despite high-dose inhalation corticosteroids		
(Severe) asthma and multiple allergies IgG-subclass deficiency discovered after immunological screening		
Internist (1st trajectory)	Internist (2nd trajectory)	Pulmonologist

Table 7.2: Continued.

Patient	The diagnostic pathway			
	Signs and symptoms	Iron deficiency anemia, recurrent lymphadenopathy and cystitis, fatigue	Recurrent respiratory infections (including proven pneumonia)/ sinusitis/ otitis	Chronic diarrhea, abdominal pain
	Attribution	Some kind of viral infection	Asthma, bacterial pneumonia	Possible diverticulitis
	Action	Follow-up	Antibiotics, inhalation corticosteroids, oral prednisolone	Referal to internist
14	Doctor	GP (1st trajectory)	Gynaecologist	GP (2nd trajectory)
	Signs and symptoms	Recurrent sinusitis and pneumonia, odontogenic infections, sepsis	Severe wound infection after cesarean section	Fatigue, exercise intolerance
	Attribution	Viral and bacterial infections	Bacterial infection	Menopause and psychological factors
	Action	Repeatedly antibiotics	Prophylactic antibiotics during second cesarean section	Referral to psychiatrist

Table 7.2. Abbreviations: CF, cystic fibrosis; CVID, common variable immunodeficiency disorders; ENT; ear-nose-throat; F, female; IgGscdef, IgG-subclass deficiency; ITP, idiopathic thrombocytopenic purpura; GP, general practitioner; M, male; n/a, not applicable; PCD, primary ciliary dyskinesia; PID, primary immunodeficiency; sIgAdef, selective IgA-deficiency; unPAD, unclassified primary antibody deficiency; XLA, X-linked agammaglobulinemia.

Chronic diarrhea, abdominal pain	Persistent abdominal pain, vomiting, recurrent respiratory infections	Persistent abdominal pain, vomiting, recurrent respiratory infections
Possible diverticulitis	n/a	CVID, possible bronchiectasis
Abdominal CT confirmed diverticulitis and kidney stones	Extensive laboratory investigations after which CVID was diagnosed, referal to pulmonologist for screening for bronchiectasis	Thymoma was coincidentally found on chest CT scan, Good syndrome was diagnosed
Psychiatrist	Patient	Immunologist
Fatigue, exercise intolerance	Recurrent sinusitis and pneumonia, odontogenic infections, sepsis, fatigue, exercise intolerance, persistent *Helicobacter Pylori* and *Giardia lamblia* infections despite treatment, recurrent cystitis, chronic slightly elevated body temperature	Recurrent sinusitis and pneumonia, odontogenic infections, sepsis, fatigue, exercise intolerance, persistent *Helicobacter Pylori* and *Giardia lamblia* infections despite treatment, recurrent cystitis, chronic slightly elevated body temperature
Psychological factors	Possible PID	Possible PID
Treatment for stress (not further specified)	Arranging own referral to immunologist	Extensive laboratory investigations after which CVID was diagnosed

DISCUSSION

This study reveals presenting patterns that can help to identify those patients who are 'always ill' and 'worn out' *with* PAD. This is important, because recurrent respiratory infections and fatigue are much more prevalent *without* concomitant PAD [22]. Participants in this study tended to normalize their symptoms and carry on with usual activities. Coping strategies of the extreme fatigue that PAD patients develop therefore differ from those with chronic fatigue syndrome, because patients with chronic fatigue syndrome often use escape/avoidance strategies [23]. Also, PAD patients reported to sleep well, whereas chronic fatigue patients generally report more difficulty falling asleep and interrupted sleep [24]. In addition to these non-medical aspects, participants recalled many medical aspects that could trigger suspicion for potential PAD: infections being unusually frequent and/or severe, not clearly season-bound, requiring antibiotics to clear. Many participants underwent repeated sinus surgery, tympanoplasty and/or polypectomy, at best only temporarily resulting in relieve of symptoms. These aspects are in fact already known, but their relevance is often missed in everyday practice. Therefore, to timely identify PAD, 'pattern recognition' should not only focus on the medical 'red flags', but also on less differentiating symptoms, such as 'being always ill' and 'worn out' and the way patients cope with these problems. And, most important, making time to really listen to the patient remains the key.

A wide range of factors affected the speed and accuracy of diagnosing PAD. First and foremost, both patients and non-immunologist healthcare professionals tended to persist in misattributing the presenting signs and symptoms to common, self-limiting illnesses or other 'innocent' explanations. Both patients and non-immunologist healthcare professionals initially attributed "being always ill" and "feeling worn out" to 'doing too much' or 'sleeping too little', or to medical conditions such as nasal polyps, nasal septum deviation, asthma or chronic rhinitis. The prevalence of most presenting symptoms of PAD in the general population is high. A population prevalence of 11% is for instance reported for sinusitis, and in a symptom prevalence study 28% of patients experienced coughing in the previous 7 days, and 11% had experienced fatigue [25,26]. This can explain why 'normalizing' prevalent symptoms such as fatigue or recurrent infections is so widespread. The normalization of symptoms and symptom misattribution to less serious or pre-existing conditions have been reported to account for appraisal delays in various cancers, particularly when the early symptoms were commonly occurring non-specific symptoms (eg, fatigue) [27,28]. Remarkably, also the appraisal of less common and more alarming signs, such as immune thrombocytopenic purpura (ITP), excessive oral aphthous lesions, recurrent meningitis, and recurrent otitis repeatedly needing tympanostomy tubes in an adult, did not alert both patients and health care professionals to potential underlying disease. Participants were referred to multiple physicians before a diagnosis was made, intensifying the time of worrying and wondering. These findings highlight an important knowledge gap among general practitioners and non-immunologist hospital doctors regarding the clinical presentation of

PAD. Education campaigns that address this issue could reduce the time between the onset of symptoms and treatment. In addition, multidisciplinary consultations (MDC's) with a number of specialists working together can support the diagnostic process for patients presenting with non-specific symptoms who have visited multiple physicians [30]. It would be interesting to explore health care professionals' views about PAD and about recognizing rare disease. This would help to reveal what health care professionals need to be able to use this knowledge in their own practice.

A second major theme was an increasing reluctance to seek care, albeit in some participants more than others. Reasons for this were diverse. They included getting so used to symptoms that they were considered to be 'normal', the feeling of not being taken seriously, and opposing 'just being treated for symptoms anyway' instead of being investigated for their cause. The quality of the doctor-patient relationship had a significant impact on the process of obtaining a correct diagnosis. A GP's prior view of a patient as being 'thin-skinned' or 'a worrier' could influence how seriously they investigated their complaints. These two themes highlight the full range of factors potentially influencing a timely diagnosis, rather than the presenting medical features of the underlying disease alone.

All this led to a significant mental burden for the participants. The period prior to the diagnosis was particularly challenging, with people feeling dismissed by health care professionals, in spite of their distress. The lack of a definitive diagnosis not only left them open to self-doubt, but also to the negative judgements of others including family, friends and employers. This concept is not unique to PAD as other conditions which are challenging to diagnose, such as systemic lupus erythematosus (SLE), elicit similar 'journeys'. In patients with SLE, the perception of being dismissed and of the lacking of empathy from a health care professional has been described as leading to feelings of emotional neglect [31]. Participants in this study reported relief when they found a name for their symptoms after they had spent years fighting searching for that. Although the PAD diagnosis helped them by legitimizing their symptoms, validating their suffering and improving their emotional status by being believed and listened to, they still had to cope with the burden of their disease and its treatment for the rest of their lives. While it has already been shown that PAD can result in a considerable disease burden [14,32], our qualitative study adds the perspective of the patients themselves. Participants found the impact of being 'always ill' and 'worn out' on their daily functioning to be a key factor determining their emotional well-being. They experienced serious limitations in their social functioning, causing social isolation and feelings of loneliness. This is in line with results from a previous study on COPD-related fatigue, which stated that fatigue influences physical, cognitive, and psychological functioning [33]. Another study related fatigue to perceived health and concluded higher fatigue correlated to a lower perceived health [34]. Other pathologies, such as different types of cancer, showed fatigue being a driver in the impact of quality of life in patients [35–37]. These results highlight the need for more attention to the potential patient burden in the diagnostic delay of PADs.

Limitations

Because of the relatively small sample, whilst the size of the sample is in keeping with the in-depth nature of qualitative research, this explorative study should perhaps be validated by future studies. The sample included patients who had been diagnosed some years previously. This may have contributed to recall bias. Nevertheless, the study produced a rich amount of material and the findings provide insight into areas of potential future research.

Conclusion

This study revealed non-medical patterns that can help to recognize patients with potential PAD. With in-depth interviewing, it became clear that – although fatigue can be one of their major complaints – these patients are different from patients with chronic fatigue syndrome: patients with PAD tend to normalize their symptoms and carry on with usual activities. The difficulty experienced by clinicians, as well as patients, in recognizing unusual and alarming signs and attributing symptoms correctly, illustrates that non-immunologists have little knowledge of PAD. This is not surprising, since PAD is a rare disease. Although this underlines the importance of education programs, which should not only focus on the medical 'red flags' of PID, but also on coping strategies of more common, less differentiating symptoms, such as 'being always ill' and 'worn out', this cannot be the final solution. It is impossible for non-experts to know about all >8,000 rare diseases. Hopefully, modern developments in automated pattern recognition can be developed to offer 'red flags' in the electronic patient file that alert a physician to potential underlying problems. The results obtained in this study can support the design of predictive models in this regard.

REFERENCES

1. Guffon N, Heron B, Chabrol B, Feillet F, Montauban V, Valayannopoulos V. Diagnosis, quality of life, and treatment of patients with Hunter syndrome in the French healthcare system: a retrospective observational study. Orphanet J Rare Dis. 2015 Apr;10:43.
2. Pierucci P, Lenato GM, Suppressa P, Lastella P, Triggiani V, Valerio R, et al. A long diagnostic delay in patients with Hereditary Haemorrhagic Telangiectasia: a questionnaire-based retrospective study. Orphanet J Rare Dis. 2012 Jun;7:33.
3. Wood P. Primary antibody deficiencies: Recognition, clinical diagnosis and referral of patients. Clinical Medicine, Journal of the Royal College of Physicians of London. 2009.
4. Gathmann B, Binder N, Ehl S, Kindle G. The European internet-based patient and research database for primary immunodeficiencies: update 2011. Clin Exp Immunol. 2012 Mar;167(3):479–91.
5. Gathmann B, Grimbacher B, Beaute J, Dudoit Y, Mahlaoui N, Fischer A, et al. The European internet-based patient and research database for primary immunodeficiencies: results 2006-2008. Clin Exp Immunol. 2009 Sep;157 Suppl:3–11.
6. Picard C, Al-Herz W, Bousfiha A, Casanova J-L, Chatila T, Conley ME, et al. Primary Immunodeficiency Diseases: an Update on the Classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015. J Clin Immunol. 2015 Nov;35(8):696–726.
7. Quinti I, Soresina A, Guerra A, Rondelli R, Spadaro G, Agostini C, et al. Effectiveness of immunoglobulin replacement therapy on clinical outcome in patients with primary antibody deficiencies: Results from a multicenter prospective cohort study. J Clin Immunol. 2011;
8. Resnick ES, Moshier EL, Godbold JH, Cunningham-Rundles C. Morbidity and mortality in common variable immune deficiency over 4 decades. Blood. 2012 Feb;119(7):1650–7.
9. de Vries E. Patient-centred screening for primary immunodeficiency, a multi-stage diagnostic protocol designed for non-immunologists: 2011 update. Clin Exp Immunol. 2012 Jan;167(1):108–19.
10. Elsink K, van Montfrans JM, van Gijn ME, Blom M, van Hagen PM, Kuijpers TW, et al. Cost and impact of early diagnosis in primary immunodeficiency disease: A literature review. Clinical Immunology. 2020.
11. Rider NL, Kutac C, Hajjar J, Scalchunes C, Seeborg FO, Boyle M, et al. Health-Related Quality of Life in Adult Patients with Common Variable Immunodeficiency Disorders and Impact of Treatment. J Clin Immunol. 2017 Jul;37(5):461–75.
12. Quinti I, Di Pietro C, Martini H, Pesce AM, Lombardi F, Baumghartner M, et al. Health related quality of life in common variable immunodeficiency. Yonsei Med J. 2012 May;53(3):603–10.
13. Sigstad HMH, Stray-Pedersen A, Frøland SS. Coping, quality of life, and hope in adults with primary antibody deficiencies. Health Qual Life Outcomes. 2005 May;3:31.
14. Berg AK, Diseth TH, Abrahamsen TG, Halvorsen K, Reinfjell T, Erichsen HC. Primary antibody deficiency: the impact on the quality of life and mental health of affected children and their parents. Acta Paediatr. 2021 Jan;
15. Peshko D, Kulbachinskaya E, Korsunskiy I, Kondrikova E, Pulvirenti F, Quinti I, et al. Health-Related Quality of Life in Children and Adults with Primary Immunodeficiencies: A Systematic Review and Meta-Analysis. J allergy Clin Immunol Pract. 2019;7(6):1929-1957.e5.
16. Tong A, Sainsbury P, Craig J. Consolidated criteria for reporting qualitative research (COREQ): a 32-item checklist for interviews and focus groups. Int J Qual Heal care J Int Soc Qual Heal Care. 2007 Dec;19(6):349–57.

17. De Vries E. Patient-centred screening for primary immunodeficiency: A multi-stage diagnostic protocol designed for non-immunologists. Clin Exp Immunol. 2006;
18. Herriot R, Sewell WAC. Antibody deficiency. Journal of Clinical Pathology. 2008.
19. Wood P. Primary antibody deficiency syndromes. Annals of Clinical Biochemistry. 2009.
20. E. De Vries and L. Nahar - Van Venrooij. Quantifying health-related well-being. 2016.
21. Gale NK, Heath G, Cameron E, Rashid S, Redwood S. Using the framework method for the analysis of qualitative data in multi-disciplinary health research - Supplementary material: Illustrative example of the use of the Framework Method. BMC Med Res Methodol. 2013;
22. Finley CR, Chan DS, Garrison S, Korownyk C, Kolber MR, Campbell S, et al. What are the most common conditions in primary care? Systematic review. Can Fam Physician. 2018;
23. Johnson SK, DeLuca J, Natelson BH. Assessing somatization disorder in the chronic fatigue Syndrome. Psychosom Med. 1996;
24. Afari N, Buchwald D. Chronic fatigue syndrome: A review. American Journal of Psychiatry. 2003.
25. Center for Health Statistics N. Table A-2. Selected respiratory diseases among adults aged 18 and over, by selected characteristics: United States, 2018. 2018.
26. Petrie KJ, Faasse K, Crichton F, Grey A. How common are symptoms? Evidence from a New Zealand national telephone survey. BMJ Open. 2014;
27. Evans J, Chapple A, Salisbury H, Corrie P, Ziebland S. "It can't be very important because it comes and goes"--patients' accounts of intermittent symptoms preceding a pancreatic cancer diagnosis: a qualitative study. BMJ Open. 2014 Feb;4(2):e004215.
28. Brouha XDR, Tromp DM, Hordijk G-J, Winnubst JAM, de Leeuw JRJ. Oral and pharyngeal cancer: analysis of patient delay at different tumor stages. Head Neck. 2005 Nov;27(11):939–45.
29. Taylor CJ, Hobbs FDR, Marshall T, Leyva-Leon F, Gale N. From breathless to failure: symptom onset and diagnostic meaning in patients with heart failure-a qualitative study. BMJ Open. 2017 Mar;7(3):e013648.
30. Barrios Y, Alonso-Larruga A, Sánchez-Machín I, Poza-Guedes P. Multidisciplinary Team Work to succeed: A Primary Immunodeficiency Unit experience. 2020;
31. Narain S, Richards HB, Satoh M, Sarmiento M, Davidson R, Shuster J, et al. Diagnostic accuracy for lupus and other systemic autoimmune diseases in the community setting. Arch Intern Med. 2004 Dec;164(22):2435–41.
32. Janssen LMA, Bassett P, Macken T, van Esch J, Pruijt H, Knoops A, et al. Mild Hypogammaglobulinemia Can Be a Serious Condition. Front Immunol. 2018;9:2384.
33. Theander K, Unosson M. Fatigue in patients with chronic obstructive pulmonary disease. J Adv Nurs. 2004.
34. Seeborg FO, Seay R, Boyle M, Boyle J, Scalchunes C, Orange JS. Perceived Health in Patients with Primary Immune Deficiency. J Clin Immunol. 2015.
35. Efficace F, Gaidano G, Breccia M, Voso MT, Cottone F, Angelucci E, et al. Prognostic value of self-reported fatigue on overall survival in patients with myelodysplastic syndromes: A multicentre, prospective, observational, cohort study. Lancet Oncol. 2015.
36. Efficace F, Baccarani M, Breccia M, Cottone F, Alimena G, Deliliers GL, et al. Chronic fatigue is the most important factor limiting health-related quality of life of chronic myeloid leukemia patients treated with imatinib. Leukemia. 2013.
37. McCabe RM, Grutsch JF, Braun DP, Nutakki SB. Fatigue as a driver of overall quality of life in cancer patients. PLoS One. 2015.

Supplementary Table 7.1. Interview questions.

Patient characteristics

General characteristics about you [your child].

- Current age: … years.
- Age at which the first symptoms started: … years.
- Age at which the diagnosis 'deficiency of antibodies' was made: … years.
- Which diagnosis do you [your child] have?
- Which treatment do you / does your child receive?
    - Immunoglobulins?
    - Antibiotic prophylaxis?

If the interviewee is not the patient himself;

- What is your relationship to the patient?
    - Father / Mother

Introduction to the patient

I would like to hear about the time when you [your child] already had complaints, but the diagnosis 'deficiency of antibodies' had not yet been made.

- How long did that period last?
    - When did the complaints start?
    - When was the diagnosis definitely made (patient age)?
- Did the symptoms start suddenly or did they develop gradually?
- What were the complaints during this period?

Together we can try to sketch a short life course. Later, we will discuss the exact complaints and what they meant for you [your child].

- Infant (birth – 18 months)
- Toddler (18 months – 3 years)
- Child (3 years – 5 years)
- Elementary school (6 years – 12 years)
- High school; adolescence (12 years – 18 years)
- Twenties, thirties, et cetera

Medical history

General and central nervous system

- Can you tell me more about your [your child's] development?
    - Was the children's healthcare center satisfied about your [your child's] development?
    - Were you [your child] able to keep up with peers?

- Was there any weight change (decrease or increase)?
- Did you [your child] often have a fever?
    - If so, how often?
    - Was there a pattern in this?
- Do you [your child] have problems with hearing and/or seeing? Or any other problems with the senses (taste, smell, touch)?
- Did you [your child] have a headache, dizziness?
- Are there any memory problems?

Cardiorespiratory
- Did you [your child] have respiratory complaints?
- How is your [your child's] exercise tolerance?
- Did you [your child] feel fatigued?
    - If so, did this have an influence on daily activities?

Digestive system
- Did you [your child] have a stomach ache?
- Were there any nutritional problems at the time?
- What was your [your child's] stool pattern?

Urogenital
- Did you [your child] have complaints when urinating?

Allergies
- Do you [your child] have allergies?
    - If so, what are you [your child] allergic to?
    - Which symptoms do you [your child] experience after contact with these allergens?
    - Is this allergy confirmed with allergy diagnostics, such as blood- or skin prick test?

Medication
- Did you [your child] use any medicines in the period before the diagnosis 'deficiency of antibodies' was made?
    - If so, for what did you [your child] use these medicines?
    - Who prescribed these medicines?
    - How long did you [your child] take these medicines?
    - Did these medicines have (the desired) effect?
- Did you [your child] use any over-the-counter medications (for example, acetaminophen, aspirin, ibuprofen, oral contraceptive pills)?

Intoxication

- Do you [your child] consume alcohol?
  - How much?
- Do you [your child] smoke?
  - How many cigarettes a day? Since when?
- Do you [your child] use drugs?

Family history

- Are there first- and/or second degree family members with similar complaints?
- Have any first- and/or second degree family members been diagnosed with an immune disorder?
- Have any family members died (because of infections)? If children died at young age, was the cause of death known?
- Is there consanguinity?

Medical history

- Have you [your child] been diagnosed with other illnesses?
- Are you [your child] being treated by a specialist in hospital?
- Have there been repeated hospitalizations?

Non-medical history

Can you tell me more about the family in which you [your child] grew up?

- Parents?
- Brothers and/or sisters?
- Special circumstances?
- Living situation?

Can you tell me more about your [your child's] school-time?

- Which school did you [your child] complete (elementary school, high school)?
- Did you [your child] receive any education?
  - If so, which education?
  - How did this go?
  - Did you [your child] experience any problems?

Can you tell me more about your further adult life?

- Which career choices did you make?
- Did the complaints have consequences for the choices you made?
  - How, what would you have done differently if you had been free of these complaints?
  - Can you tell me more about that?

Can you tell me more about your [your child's] leisure activities?

- Do you [your child] practice a sport?
  - If so, which sport?
  - Performances?
- What are your [your child's] hobbies?

Can you tell me more about your [your child's] social life?

- Do you [your child] have friends?
- Do you [your child] ever go out?
- Did your [your child's] complaints entail restrictions?
- Do you [your child] have enough energy to undertake activities?

Supplementary Table 7.2. Codes, categories and themes used in the qualitative analysis.

Theme	Category	Code
PRE-DIAGNOSIS: JOURNEY TO REFERRAL	**Self-reported presenting symptoms**	- (Recurrent) fever - Abdominal cramps - Abnormal stool pattern - Arthralgia - Back complaints - Chronically slightly elevated body temperature - Cough - Development of speech - Diarrhea - Dyspnea - Dizziness - Exercise intolerance - Fatigue - Feeling ill - Forgetful - Growth problem - Hair loss - Headache - Hospital admission - Impaired sense of smell - Impaired sense of taste - Lymphadenopathy - Muscle pains - Nasal polyps - Nausea - Night sweats - Overall malaise - Pain - Palpitations - Reduced vision - Stomach and bowel complaints - Swallowing - Tonsillectomy - Tympanostomy - Vomiting - Weight gain - Weight loss

Supplementary Table 7.2. Codes, categories and themes used in the qualitative analysis.

Theme	Category	Code
	Self-reported presenting clinical manifestations	- Abscesses - Alopecia areata - Allergic reaction to influenza vaccination - Allergic reaction(s) - Aphthous lesions - Aphthous stomatitis - Anemia - Arthrosis - Asthma - Bronchitis - Bronchiectasis - Cataract - Chronic hives - Chronic otitis - Chronic sinusitis - Chronic dermatitis - Contact dermatitis - Eczema - Erythema nodosum - Food hypersensitivity - 'Flu' - Gastritis - GLILD - Graves' disease - Growth retardation - Headaches/migraine - Hepatitis - Hip injury - Hypertension - Hypothyroidism - Iron deficiency - ITP - 'Kind of flu that does not really break through' - Laryngitis - Mucoepidermoid carcinoma - Non-responder to hepatitis A/B vaccination - Peritonitis - Pharyngitis - Pneumonia - Pyelonephritis - Recurrent typical childhood diseases - Recurrent cystitis - Recurrent lower respiratory tract infections

Supplementary Table 7.2. Codes, categories and themes used in the qualitative analysis.

Theme	Category	Code
		- Recurrent otitis - Recurrent meningitis - Recurrent rhinitis - Recurrent sinusitis - Recurrent pharyngitis/tonsillitis - Respiratory tract infections (not further specified) - Salpingitis - Sclerosis - Splenomegaly - Urticaria - Warts
	Time pattern in clinical manifestations	- Impact of seasons - Pattern of signs and symptoms - 'Life line' of signs and symptoms
	Symptom appraisal	- Interpretation of signs and symptoms by patients - Interpretation of signs and symptoms by the social environment - Interpretation of signs and symptoms by the consulting doctor - 'Doctors don't know'
	Emotional toll of the diagnostic process	- Impact of symptoms on future perspectives - Impact of symptoms on mental well being - Impact of symptoms on relationships - Impact of symptoms on quality of life - Impact of symptoms on regular activities - Battle for legitimacy - The time it took to receive a correct diagnosis - Losing confidence in the healthcare system
	Coping with symptoms	- Fighting against symptoms - Trivializing symptoms - Adjustments in daily life - Role of the social environment
	Family history	- Family member with the same diagnosis - Family member with symptoms suggesting primary immunodeficiency, but not investigated - Family member passed away at young age - Family member passed away because of infections

Supplementary Table 7.2. Codes, categories and themes used in the qualitative analysis.

Theme	Category	Code
DIAGNOSIS: EXPERIENCE OF RECEIVING THE DIAGNOSIS	**Care-seeking**	- The cue to seek help - The cue for the doctor to conduct further investigations - The patient journey - Fighting for right referrals and pre-diagnosis treatment - Shared decision-making regarding diagnostic analysis / referral - Person who made/suggested the diagnosis - The way the PAD diagnosis was communicated by doctors - Reaction to receiving a PAD diagnosis - Explanation of the diagnosis by the patient - Patient's view on reducing diagnostic delay
POST-DIAGNOSIS: IMPACT OF THE DIAGNOSIS	**Issues relating to care-provision**	- Care after the PAD diagnosis - The speed of initial treatment - Shared decision-making regarding treatment - Perceived effect of treatment by patient - Patient autonomy - Patient expertise - Retrospective patient view on care-provision - Disagreement between different doctors - Knowledge about PAD by doctors
	Impact of the diagnosis and/or treatment	- Impact of illness and/or treatment on patient's own and family's activities - Impact of illness on being able to work - Post-diagnosis stress / frustrations - Treatment burden - Complications discovered after diagnosis
	Coping with the diagnosis	- Adjustments in daily life - Support from the social environment - Developing resilience

Chapter 8

Focusing on good responders to pneumococcal polysaccharide vaccination in general hospital patients suspected for immunodeficiency. A decision tree based on the 23-valent Pneumococcal IgG assay

Lisanne M.A. Janssen, Michiel Heron, Jean-Luc Murk, Alexander C.A.P. Leenders, Ger T. Rijkers, Esther de Vries

Front Immunol. 2019; 10:2496

ABSTRACT

Background & Aim

Recently, the 23-valent IgG-assay was suggested as screening assay to identify *poor* responders to pneumococcal polysaccharide (PnPS)-vaccination with the serotype-specific assay as a second-line test. However, in a low pre-test probability general hospital setting predicting *good* responders could be more valuable to reduce the number of samples needing serotyping.

Methods

Serotype-specific PnPS antibody-assays were performed for suspected immunodeficiency in two Dutch general hospitals (Jeroen Bosch Hospital, 's-Hertogenbosch; Elisabeth Tweesteden Hospital, Tilburg). 23-valent PnPS antibody-assays were subsequently performed in archived material. Data were analysed using receiver operating characteristic curves (AUC) and agreement indices (ICC).

Results

Sera of 284 patients (348 samples) were included; 23-valent IgG-titres and the corresponding sum of PnPS-serotype specific antibodies showed moderate correlation (ICC=0.63). In 232 conjugated-pneumococcal-vaccine-naïve patients (270 samples), a random 23-valent IgG-titre could discriminate between samples with and without ≥7/11, ≥7/13 or ≥ 6/9 pneumococcal serotypes when both cut-off values 0.35 and 1.0 μg/ml were used (AUC 0.86 and 0.92, respectively). All patients with a pre-immunisation-titre ≥38.2 μg/ml and/or post-immunisation-titre ≥96.1 μg/ml and none with a post-immunisation-titre ≤38.5 μg/ml exhibited a good response to PnPS vaccination. Using these breakpoints as screening test to predict *good* responders, only 24% of patients would require further serotyping, as opposed to 68% if breakpoints to predict *poor* responders would have been used.

Conclusion

In a low pre-test probability setting, the 23-valent IgG-assay proved to be a reliable screening test for good responders in conjugated-pneumococcal-vaccine-naïve patients, reducing the overall number of patient samples needing further serotyping, thus reducing overall costs of pneumococcal vaccination response assessment.

INTRODUCTION

Serotype-specific pneumococcal polysaccharide (PnPS) antibody testing is currently accepted as the 'gold standard' [1–4] for the evaluation of anti-polysaccharide antibody production capacity in patients who are suspected to have primary antibody deficiency because of unexplained or recurrent (mainly respiratory) infections [4,5]. However, serotype-specific PnPS testing is not widely available and is time consuming, labour intensive and expensive. Moreover, uniform reference values are not available, and interpretation is therefore challenging [6–10].

Recent data has indicated that one-step measurement of the summated response to all 23 serotypes present in the polysaccharide pneumococcal vaccine (here called "23-valent IgG assay") could be used as a screening test to reduce the overall number of patient samples needing serotyping [11,12]. This could significantly improve efficiency and reduce overall costs. In addition, this assay is widely available as in-house assay or easy-to-use commercial kit, and the test result is easy to interpret based on a single cut-off value [13]. Given these advantages, the 23-valent IgG assay has been proposed to be used as a first-line test to identify clear-cut poor responders, and the serotype-specific assay as a second-line test for assessment of the PnPS vaccination response in non-clear-cut cases only. In their tertiary-centre adult cohort (n=62), Lopez et al. identified a cut-off value of 110 μg/ml, which was constantly associated with a poor response to PnPS vaccination using the serotype-specific assay [11].

However, *on a population basis* - i.e. in the context of a low pre-test probability setting - a screening method that can reliably predict *good* responders could be of greater value. After all, many patients with recurrent infections do not have an immunodeficiency. Or they suffer from milder forms of hypogammaglobulinemia such as selective anti-polysaccharide antibody deficiency (SPAD) only (or combinations with IgG-subclass and/or IgA deficiency), without significantly decreased total immunoglobulin levels. These patients generally present themselves in secondary care, where the pre-test probability for severe antibody deficiency is inherently low. However, even milder hypogammaglobulinemia can lead to serious problems, requiring adequate medical attention [14]. These milder patients are often not recognized due to lack of available test facilities in secondary care, and reluctance to refer many patients to an immunologist. Easy, reliable selection of patients can create support for a lower screening threshold for antibody deficiency in patients with recurrent infections in secondary care. Ultimately, this will help timely detection of all patients who do have an immunodeficiency. Our study was designed to investigate the suitability of the one-step summated response test for this purpose.

MATERIAL AND METHODS

Study design

Between February 2012 and December 2018, serotype-specific PnPS assays were performed on 348 blood samples in regular patient care, obtained from 284 patients who were analysed for potential immunodeficiency in two secondary centres in the Netherlands (Jeroen Bosch Hospital, 's-Hertogenbosch (n=234), Elisabeth Tweesteden Hospital, Tilburg (n=50)). Of these, 78 samples were from 64 patients who were previously vaccinated with conjugated pneumococcal vaccine (Pn-C). Left-over samples were stored at ≤ -80 °C and later retrieved from the laboratory to perform 23-valent pneumococcal IgG assays. The research project was granted ethical approval by the local medical ethics committee and consent was obtained from all adults and parents of the children.

Test methods

The Clinical Reference Standard

The IgG antibodies against PnPS were measured on a Luminex platform using a quantitative multiplex immunoassay including cell wall polysaccharide (CPS) and 22F adsorption [15]. For the Jeroen Bosch Hospital, this serotype-specific assay was performed in the Department of Medical Immunology, University Medical Centre Utrecht, the Netherlands. Titters were assessed against eleven serotypes (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F) until February 2014, and thereafter against nine serotypes (6B, 8, 9V, 14, 15B, 19F, 20, 23F, 33F). For the Elisabeth Tweesteden Hospital, this assay was performed in the St. Antonius Hospital, Nieuwegein, the Netherlands. In this laboratory, titres were assessed against thirteen serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F); in a subset of these samples (n=132), 22 of the serotypes present in the 23-valent IgG assay (all except 17F) were determined. For the interpretation of PnPS serotype concentrations two different thresholds were used: ≥ 0.35 and ≥ 1.0 µg/ml (based on protection against invasive infection and colonization, respectively) [10,16–19]. For both limits, sufficient levels were defined in vaccine-naïve patients as ≥ 7/11, ≥ 7/13 or ≥ 6/9 serotypes reaching these concentrations (based on the reference values of the respective laboratories). In 174/284 (61%) patients a blood sample was drawn 4-8 weeks after intramuscular vaccination with one dose of 23-valent PnPS vaccine (Pneumovax 23; Merck, Sharp & Dohme BV, Haarlem, The Netherlands) containing 23 µg purified type-specific capsular polysaccharide of 23 pneumococcal serotypes (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F; Danish nomenclature). A positive response to PnPS vaccination was defined according to the guidelines of the laboratory (17,18). Briefly, a good response to PnPS vaccination was defined by a post-immunization titre ≥ 1.0 µg/ml in ≥ 7/11, ≥ 7/13 or ≥ 6/9 serotypes in patients not previously immunized with Pn-C vaccine. In Pn-C pre-vaccinated patients, the serotypes not present in the vaccine were evaluated (Utrecht; when vaccinated with Pn-C7, serotypes 1, 3, 5, 7F; when vaccinated with

Pn-C10, serotypes 8, 15B, 20, 33F | Nieuwegein; when vaccinated with Pn-C7, serotypes 1, 3, 5, 6A, 7F, 19A; when vaccinated with Pn-C10, serotypes 3, 6A, 19A). According to the Utrecht laboratory's reference values, we corrected for age in these samples: for ages 4-6 years, an abnormal result was defined as < 50% of serotypes evaluated reaching an IgG titre of ≥ 1 μg/ml. For age ≥6 years, an abnormal result was defined as <75% of serotypes evaluated reaching an IgG titre of ≥ 1 μg/ml. According to the Nieuwegein laboratory's reference values, an abnormal result was defined as < 70% of serotypes evaluated reaching an IgG titre of ≥ 1 μg/ml in those samples.

The Index Test

For the measurement of the 23-valent IgG titre, the VaccZyme™ anti-PCP IgG ELISA Kit (The Binding Site, Birmingham, United Kingdom) with precoated microtiter plates was used according to the manufacturer's instructions [22,23]. Absorption of interfering anti-cell wall polysaccharide (CPS) antibodies was incorporated in this assay. The VaccZyme™ anti-PCP IgG assay was performed in the Laboratory of Medical Microbiology and Immunology at the Elisabeth Tweesteden Hospital (Tilburg, the Netherlands).

Statistical analysis

Data were analysed using SPSS 24.0 software for Mac. Differences in measurements were tested with t test (Welch's t test when the variances are unequal) and ANOVA. Separate analyses were performed for patients previously immunized with Pn-C. Correlation between the 23-valent IgG titre and the sum of the serotype-specific antibody titres in the same sample was assessed with the intraclass correlation coefficient (ICC). The strength of the relationship for the ICC coefficient r was classified as follows: 0.3 ≤ r < 0.5 'poor', 0.5 ≤ r < 0.75 'moderate', 0.75 ≤ r < 0.9 'good' and 0.9 ≤ r < 1.0 'excellent' [24]. To determine whether a random 23-valent IgG titre could predict that ≥ 7/11, ≥ 7/13 or ≥ 6/9 (vaccine-naïve patients) vs ≥ 2-3/4, ≥ 2/3 or ≥ 4/6 (Pn-C pre-vaccinated patients) serotypes were above the two different cut-off levels (≥0.35 or ≥1.0 μg/ml) in the same sample, receiver operating characteristics (ROC) curves were plotted and the area under the curves (AUCs) were calculated. To determine whether the pre-, or 4-8 weeks post-immunization 23-valent IgG titre could predict if that patient would become a good or poor responder to PnPS vaccination as assessed by the serotype-specific assay, also for these variables ROC curves were plotted and AUCs calculated. The best cut-off values were chosen according to 1) the Youden index calculation, 2) the maximum sensitivity for pre-immunization 23-valent IgG titres, and 3) the maximum sensitivity and specificity for 4-8w weeks post-immunization 23-valent IgG titres. For each identified cut-off value, the positive and negative predictive values were calculated. All tests were two-tailed and p-values < 0.05 were considered to be statistically significant.

RESULTS

Participants

127/284 (45%) patients were females, and the mean age at inclusion was 36.3 years (range 1.1-89.7). In 54 patients, two or more different samples were available, resulting in 348 samples with paired serotype-specific and 23-valent pneumococcal IgG titres available for analysis (78/348 (22%) samples from patients previously immunized with Pn-C vaccine). Of the 270 samples from patients not previously immunized with Pn-C vaccine, 194 were pre-immunization samples, 38 were 4-8 weeks post-immunization samples, and 38 were > 8 weeks post-immunization samples (mean duration after vaccination 33 months, range 15-70 months). Of the 78 samples from patients previously immunized with Pn-C vaccine, 63 were pre-immunization samples, 14 were 4-8 weeks post-immunization samples, and 1 was a > 8 weeks post-immunization sample (28 months).

Test results

In all samples taken together, a moderate correlation between the sum of the individual PnPS serotypes and the 23-valent IgG titre in the same sample was observed (ICC = 0.65, 95% CI = 0.57-0.72, P < 0.0001; **Figure 8.1A**). The ICC did not improve when the sum of a larger set of 22 individual PnPS serotypes was plotted against the 23-valent IgG titre (available for 132 samples; ICC = 0.64, 95% CI = 0.49-0.75; **Figure 8.1B**).

Patients not previously immunized with conjugated pneumococcal vaccine

First, all samples from patients not previously immunized with conjugated pneumococcal vaccine were analysed together, irrespective of whether the patients had received PnPS-vaccination. The 23-valent IgG titres were significantly higher in samples with ≥ 7/11, ≥ 7/13 or ≥ 6/9 serotypes above both the cut-off levels 0.35 and 1.0 µg/ml, respectively, compared to samples with < 7/11, < 7/13 or < 6/9 serotypes above these cut-off levels (p < 0.0001 for both cut-off levels; **Figure 8.2**). A 23-valent IgG titre could discriminate between samples with and without ≥7/11, ≥7/13 or ≥ 6/9 serotypes above both the cut-off values 0.35 and 1.0 µg/ml [ROC analysis; AUC 0.86 (95% CI 0.82-0.91) and 0.92 (95% CI 0.88-0.95), respectively; **Figure 8.2**, **Table 8.1**]. Based on the calculation of the Youden index, the best threshold was a 23-valent IgG titre of ≤ 38.2 µg/ml for the serotype-specific cut-off level of 0.35 µg/ml and ≤ 54.2 µg/ml for the cut-off level 1.0 µg/ml. However, neither of them achieved estimates of both sensitivity and specificity greater than 86% (**Table 8.1**).

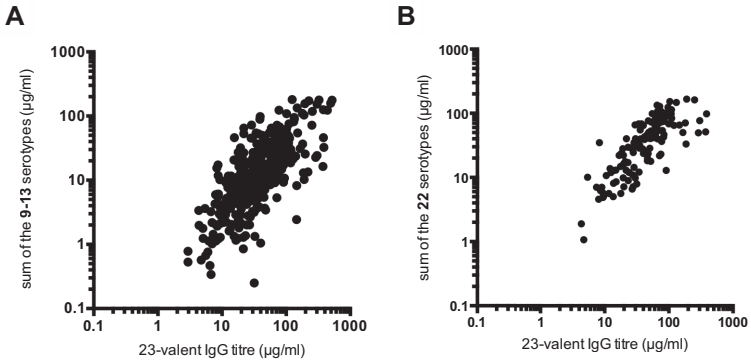


Figure 8.1. Correlation between the 23-valent pneumococcal IgG titre and the sum of the pneumococcal polysaccharide serotype titres determined in the same sample.

Figure 8.1. A: sum of 9-13 individual serotypes, 348 samples, ICC = 0.65, 95% CI = 0.57-0.72, P< 0.0001; B: sum of 22 individual serotypes, 132 samples, ICC = 0.64, 95% CI = 0.49-0.75.

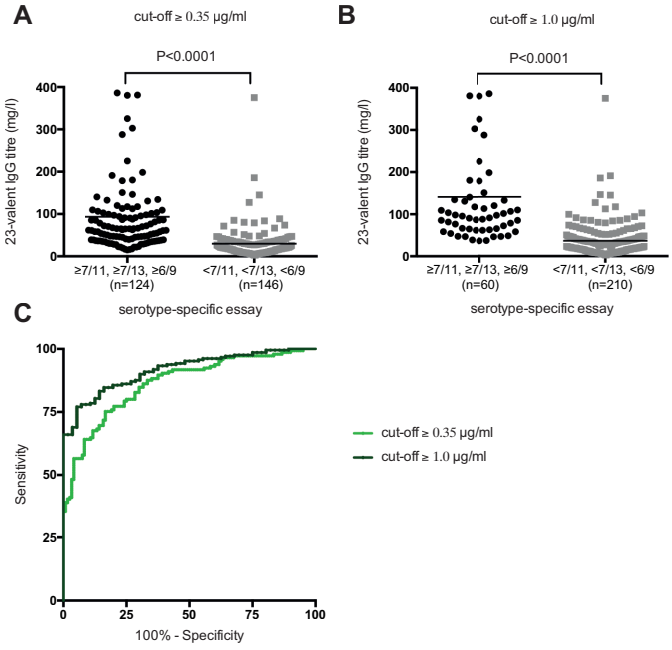


Figure 8.2. 23-valent pneumococcal IgG titres in samples with and samples without ≥ 7/11, ≥ 7/13 or ≥ 6/9 serotypes above the cut-off values of 0.35 µg/ml (A) and 1.0 µg/ml (B).

Figure 8.2. P-values were calculated in an unpaired T-test. Receiver operating characteristic (ROC) curves of sensitivity versus specificity of the 23-valent pneumococcal IgG titre using two different cut-off levels for the serotype-specific assay (C).

Table 8.1. 23-valent pneumococcal IgG titres compared to the serotype-specific assay in the same sample (n=270).

A. Estimated areas under the curve (AUCs) with their (95% CI) and p-values.			
	AUC (95%CI)	**P-value**	**Youden Index**
Cut-off ≥0.35 µg/ml	0.86 (0.82-0.91)	<0.0001	0.55
Cut-off ≥1.0 µg/ml	0.92 (0.88-0.95)	<0.0001	0.69

B. Performance indicators and levels of agreement for the 23-valent pneumococcal IgG titres.			
Criterion	**Se (%)**	**Sp (%)**	**PPV (%)**
Cut-off value 0.35 ug/ml			
≤38.2[1]	79.3 (71.8-85.6)	75.8 (67.2-83.2)	79.3 (73.5-84.1)
Cut-off value 1.0 ug/ml			
≤54.2[1]	83.3 (77.5-88.1)	85.7 (73.8-93.6)	95.6 (91.9-97.7)

Table 8.1. [1]Selected using the Youden index. All statistics are presented with the corresponding (95% CI). Abbreviations: CI, confidence interval; IgG, immunoglobulin G; NPV, negative predictive value; PPV, positive predictive value; Se, sensitivity; Sp, specificity.

Next, the pre- and 4-8 weeks post-immunization 23-valent IgG measurements were compared with the serotype-specific PnPS vaccination response to perform a *per patient* analysis (information available for 147 patients). This is clinically the most interesting evaluation. Sixty (41%) patients were defined as poor responders according to the serotype-specific assay. For both 23-valent pre- and 4-8 weeks post-immunization titres a significant difference was observed between good and poor responders ($p < 0.0001$; **Figure 8.3**). The results of ROC curve analyses of the 23-valent IgG assay performance vs. the serotype-specific assay per patient are shown in **Figure 8.3** and **Table 8.2**.

Because Cohen's kappa was never better than moderate (<0.75), this ruled out the use of the 23-valent IgG assay alone and implicated the necessity of a stepwise approach. We wanted to establish whether use of the 23-valent IgG assay alone could reliably discriminate good responders, who do not need any further diagnostic work-up, as well as poor responders, whom we do not want to miss. We therefore favoured a high sensitivity for *pre*-immunization titres, and both high specificity as well as high sensitivity for *post*-immunization titres. All patients with a 23-valent pre-immunization titre ≥ 38.2 µg/ml and/or post-immunization titre ≥ 96.1 µg/ml and none of the patients with a post-immunization titre ≤ 38.5 µg/ml exhibited a good response to PnPS vaccination (**Table 8.2B**).

Best-choice criterion according to:		
Youden Index calculation	**100% Se**	**100% Sp**
≤ 38.2 µg/ml	≥188.5 µg/ml	≤16.1 µg/ml
≤ 54.2 µg/ml	≥188.5 µg/ml	≤36.8 µg/ml

NPV (%)	**McNemar's test p-value**	**Cohen's kappa**
75.2 (68.6-80.8)	1.000	0.55
59.1 (51.4-66.4)	< 0.0001	0.60

Based on these data, a stepwise approach was developed using both tests (**Figure 8.4**). 24% (26/109) of patients had a pre-immunization 23-valent IgG level ≥ 38.2 µg/ml, 32% (12/38) of patients had a post-immunization 23-valent IgG level ≤ 38.5 µg/ml; 37% (14/38) of patients had a post-immunization 23-valent IgG level ≥ 96.1 µg/ml (in four of these patients also a pre-immunization sample was available). A scenario using the 23-valent IgG assay as pre-screen would have decreased costs in our general hospital patient population from $46,00 down to $20,37 per patient (based on global average test prices of the 23-valent IgG assay of $5.30 per well, and of the serotype-specific assay of $46.00 per sample, and on the assumption that all wells are used in the test run; calculation: (100 x 5,30 + 76 x 5,30 + 24 x 46,00)/100 = 20,37) [25].

Antibody levels against individual serotypes differed considerably, with serotypes 14 and 19F being dominant in most cases (**Figure 8.5**). On average, antibodies to individual serotypes contributed to the total 23-valent IgG titre ranging from 1.1 – 7.6% in pre-immunization samples, and 1.1 – 14.7% in post-immunization samples. In pre-immunization samples, serotype 14 contributed significantly more to the 23-valent IgG titre compared to serotypes 1, 3, 4, 5, 6A, 6B, 7, 9, 18C, and 23F (analysis of variance; $F = 5.974$, $p = < 0.0001$). In post-immunization samples, no overall dominant serotype could be identified (analysis of variance; $F = 1.291$, $p = 0.20$). In 19 pre-immunization and 4 post-immunization samples, antibodies against a single serotype contributed more than 50% to the 23-valent IgG titre, but

only two of those pre-immunization samples and none of those post-immunization samples had a high 23-valent IgG titre (defined as ≥ 38.2 µg/ml pre-immunization and ≥96.1 µg/ml post-immunization, **Supplementary Figure 8.1**). In these two samples, the pre-immunization sample where serotype 19F dominated (73% of the 23-valent IgG titre), had 4/13 serotypes with a concentration ≥ 0.35 µg/ml and 2/13 serotypes ≥1.0 µg/ml. A post-immunisation sample was not available for this patient, therefore it was not possible to classify this patient as good or bad responder to PnPS vaccination. In the pre-immunization sample where serotype 14 was dominant (84% of the 23-valent IgG titre), an additional 7/9 serotypes had concentrations above both cut-off levels 0.35 and 1.0 µg/ml (this patient can be considered a good responder based on natural exposure).

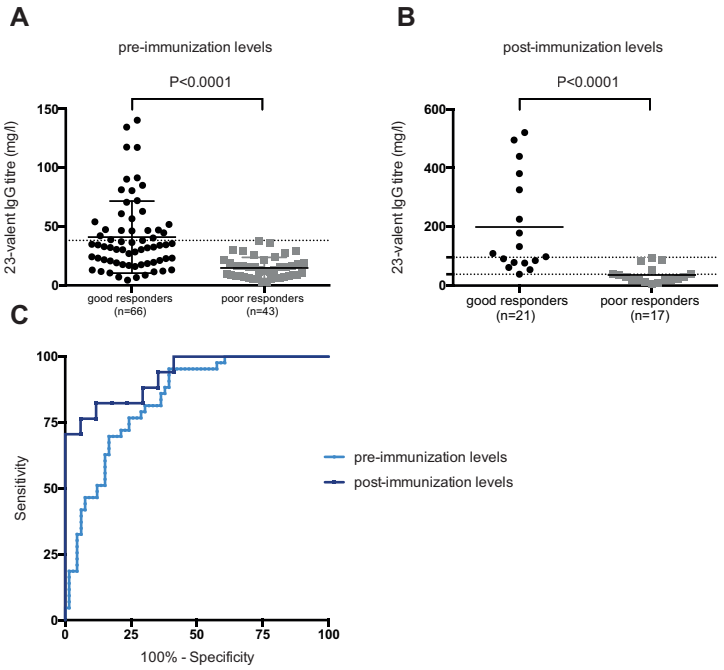


Figure 8.3. 23-valent pneumococcal IgG titres for good vs. poor responders.

Figure 8.3. Two indicated parameters were used in a per patient analysis: (A) 23-valent pre-immunization IgG levels, (B) 23-valent post-immunization IgG levels in good- and poor responders to pneumococcal polysaccharide vaccination. P-values were calculated in an unpaired T-test. Receiver operating characteristic (ROC) curves of sensitivity versus specificity for the 23-valent pre- and 4-8 weeks post-immunization levels versus serotype-specific response to vaccination (C).

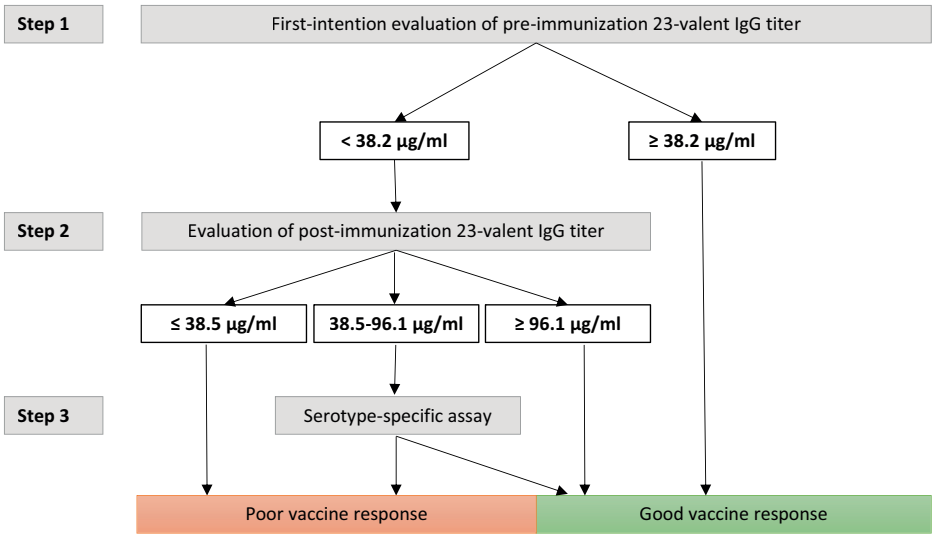


Figure 8.4. Decision tree.

Figure 8.4. Decision tree using the 23-valent pre-immunization titre as a first-line test, 23-valent 4-8 weeks post-immunization titre as second-line test, and serotype-specific assay for definitive assessment of response, if indicated. The cut-off levels were determined only on patients who were not previously immunized with conjugated pneumococcal vaccine (see Results section and Figure 8.3).

Patients previously immunized with conjugated pneumococcal vaccine

First, all samples from patients who were previously immunized with conjugated pneumococcal vaccine were analysed together (n=78), irrespective of whether the patients had received PnPS-vaccination. The 23-valent IgG titres were higher in samples with ≥ 2-3/4, ≥ 2/3 or ≥ 4/6 serotypes above both the cut-off levels of 0.35 and 1.0 µg/ml, respectively, compared to the samples with < 2-3/4, < 2/3 or < 4/6 serotypes above these cut-off levels (p=0.013 for the cut-off level of 0.35 µg/ml, and p=0.058 for the cut-off level of 1.0 µg/ml; **Supplementary Figure 8.2**). The 23-valent IgG titre could only fairly discriminate between samples with and without ≥ 2-3/4, ≥ 2/3 or ≥ 4/6 serotypes above both the cut-off values 0.35 and 1.0 µg/ml [ROC analysis; AUC 0.77 (95% CI 0.66-0.88) and 0.80 (95% CI 0.67-0.92), respectively; **Supplementary Figure 8.1**]. Based on the calculation of the Youden index, the best threshold was a 23-valent IgG titre of ≤ 50.2 µg/ml for the serotype-specific cut-off level of 0.35 µg/ml (sensitivity 76%, specificity 71%) and ≤ 58.4 µg/ml for the cut-off level 1.0 µg/ml (sensitivity 77%, specificity 83%).

Table 8.2. 23-valent pneumococcal IgG titres compared to serotype-specific response to pneumococcal vaccination in patients not previously immunized with conjugated pneumococcal vaccine.

A. Estimated areas under the curve (AUCs) with their (95% CI) and p-values.

	AUC (95%CI)	P-value	Youden Index
Pre-immunization levels (n=109)	0.84 (0.76-0.91)	<0.0001	0.51
4-8 weeks post-immunization levels (n=38)	0.93 (0.85-1.00)	<0.0001	0.71

B. Performance indicators and levels of agreement for the 23-valent IgG titres.

Criterion	Se (%)	Sp (%)	PPV (%)
Pre-immunization titre (µg/ml)			
≤ 22.4[1]	81.4 (66.6-91.6)	69.7 (57.2-80.4)	63.6 (54.2-72.2)
≥ 38.2	100.0 (91.8-100.0)	39.9 (27.6-52.2)	48.2 (43.6-52.8)
4-8 weeks post-immunization titre (µg/ml)			
≤ 58.3[1]	82.4 (56.6-96.2)	88.2 (63.6-98.5)	87.5 (64.8-96.4)
≤ 38.5	70.6 (44.0-89.7)	100.0 (80.5-100.0)	100.0
≥ 96.1	100.0 (80.5-100.0)	58.8 (32.9-81.6)	66.7 (53.5-77.7)

Table 8.2. [1]Selected using the Youden index. The selected threshold's performance is highlighted in bold front. All statistics are presented with the corresponding (95% CI).
Abbreviations: CI, confidence interval; IgG, immunoglobulin G; NC, not calculated; NPV, negative predictive value; PPV, positive predictive value; Se, sensitivity; Sp, specificity.

Next, the pre- and 4-8 weeks post-immunization 23-valent IgG measurements were compared with the serotype-specific PnPS vaccination response to perform a *per patient* analysis (information available for 27 patients; 33% (26/78) of patients were < 4 years and therefore not yet tested with PnPS vaccination). Eight (30%) patients were defined as poor responders according to the serotype-specific assay. For both 23-valent pre- and 4-8 weeks post-immunization titres, there was no significant difference between good and poor responders (pre-immunization titres: mean 43.8 µg/ml vs. 43.0 µg/ml, p=0.584; post-immunization titres: mean 157.2 µg/ml vs. 148.0 µg/ml, p=0.401). Because too few patients in our cohort were vaccinated with the 23-valent PnPS vaccine, test performance statistics could not be performed on these data.

	Best-choice criterion according to:		
	Youden Index calculation	**100% Se**	**100% Sp**
	≤ 22.4 µg/ml	≥ 38.2 µg/ml	NC
	≤ 58.3 µg/ml	≥ 96.1 µg/ml	≤ 38.5 µg/ml

	NPV (%)	**McNemar's test p-value**	**Cohen's kappa**
	85.2 (75.1-91.6)	0.003	0.44
	100.0	< 0.0001	0.34
	86.4 (69.2-94.7)	1.000	0.73
	80.8 (66.8-89.8)	0.063	0.73
	100.0	0.016	0.64

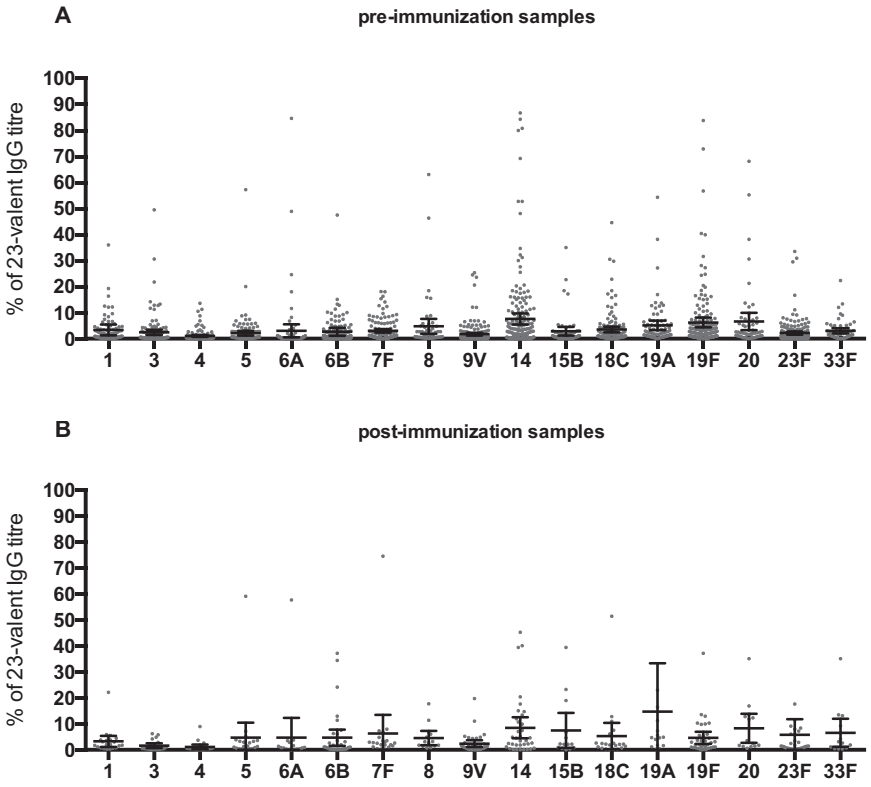


Figure 8.5. Percentage that a serotype contributed to the 23-valent IgG titre.

Figure 8.5. A. pre-immunization samples, and B. post-immunization samples in Pn-C vaccine-naïve patients. Error bars represent mean percentages + 95%CI.

DISCUSSION

We showed that a 23-valent IgG assay can be a reliable screening test to predict *good* responders to PnPS-vaccination in conjugated-vaccine-naïve patients in the low pre-test probability setting of a general hospital using our decision tree (**Figure 8.4**). The 23-valent IgG assay is widely available and easy to interpret. Implementing this procedure in general hospital care could lower the threshold for timely detection of primary antibody deficiency (PAD) by reducing the overall number of patient samples needing serotype specific antibody measurement, thus reducing overall costs. It is important to realize that recent studies focusing on using this assay as a first-line test were used to screen for *poor* responders to PnPS vaccination in highly selected patient populations referred to tertiary immunodeficiency expert centres [11,12]. Both approaches are valuable, but each should be used in the appropriate setting only.

We found that a cut-off value of ≥ 38.2 μg/ml in the pre-PnPS-immunization 23-valent IgG assay could reliably predict good responders in our cohort of conjugated-vaccine-naïve patients. This cut-off value is higher than the lower limit of the normal range (10.0, 11.0, and 15.4 μg/ml), and just below the means (41.0, 45.8, and 59.5 μg/ml) found in 3 previous studies of healthy vaccine-naïve adults using the same assay as our study (13,23,25). A post-PnPS-immunization threshold of ≤ 38.5 μg/ml yielded 100% specificity in conjugated-vaccine-naïve patients. This cut-off value is below the lower limit of the normal range post-vaccination (50 and 77 μg/ml) found in 2 studies of healthy unvaccinated adults using the same assay as our study [27,29], and similar to the cut-off value (≤ 40 μg/ml) used by the Utrecht group [12,22]. It is also lower than found in a previous study by Lopez et al, were a 23-valent IgG titre of ≤ 110 μg/ml yielded a specificity of 100% and predicted 57% of the poor responders to PnPS vaccination [11]. However, our data are not easily comparable to those of Lopez et al., as different sets and numbers of serotypes were tested in the serotype-specific assay, the criteria to define a deficient response were different, and, most important, they investigated a highly selected patient population of which 75% was diagnosed with a humoral immunodeficiency. In contrast, good responders predominated in our general population cohort. This again emphasizes the importance of fitting the screening approach to the appropriate setting.

The performance of the 23-valent IgG assay was better in post-immunization 23-valent IgG sera (AUC 0.93) than in pre-immunization sera (AUC 0.84), which is consistent with previous studies [11,28,30].

In Pn-C pre-vaccinated patients, both pre- and post-23-valent IgG titres were similar in good and poor responders. This is in agreement with previous studies, in which it has been shown that the 23-valent IgG assay could not discriminate between good and poor responders to PnPS-vaccination in Pn-C pre-vaccinated patients [12,31]. Since a majority of childhood vaccination programs now include Pn-C vaccination, and a 20-valent conjugated vaccine is currently investigated with the intent to broaden global protection against pneumococcal

disease, the future value of the 23-valent IgG assay as screening method for SPAD will probably become limited. Recent results show promising results for the measurement of the Typhim Vi IgG response as a diagnostic tool for assessing polysaccharide production in Pn-C pre-vaccinated patients [32].

The WHO recommended assay for measuring serotype-specific PnPS antibodies is by ELISA. A growing number of clinical laboratories, including ours, now are using multiplex bead technology for this purpose. The correlation between the two types of assays is good, although there can be variation in the absolute concentrations measured. It has been evaluated whether this variation would affect response classification of patients when using paired clinical sera [33]. It was concluded that despite variation in absolute values of pneumococcal antibodies, the overall classification of the pneumococcal immune status of the patient was remarkably similar between assays [33,34]. In a recent publication it has been suggested to adjust multiplex cut-off values of selected polysaccharides to improve agreement level with WHO ELISA [35].

Our study has several limitations. First, a high 23-valent IgG titre corresponding with a good PnPS vaccination response by the serotype-specific assay may nevertheless not be protective because the antibody has low avidity or low opsonophagocytic activity. However, there are currently no accepted clinical criteria regarding normal PnPS-vaccination response based on measurements other than serotype-specific PnPS concentration [3]. For future studies, it would be interesting to compare the 23-valent IgG assay with functional opsonophagocytosis assays. Second, the modest correlation between the 23-valent IgG titre and the sum of individual PnPS serotypes may be explained by the use of serotype-specific results expressed as 'higher than' (for example >10 μg/ml). Determination of the exact concentration by titration of the sera, which has not been performed in this study, may result in a better agreement between both assays. It has long been recognized that the 23-valent IgG assay is of limited value in patients where isolated, elevated serotype titres are responsible for a high 23-valent IgG titre. While this may be true for selected patient cohorts, in a general patient population such as ours, this turns out not to be the case. In only few samples (19/270 pre-immunization and 4/38 post-immunization), an isolated serotype contributed more than 50% to the 23-valent IgG titre, and a high 23-valent IgG titre was never present in the four post-immunization samples and only in 2/19 pre-immunization samples. Unfortunately, these two patients were not vaccinated, so it cannot be excluded that they could be incorrectly classified as 'expected to be a good responder' by our decision tree. Last, it should be pointed out that in our per patient analysis, post-immunization samples obtained > 8 weeks after vaccination were excluded. The number of those samples was too low to perform a meaningful comparison.

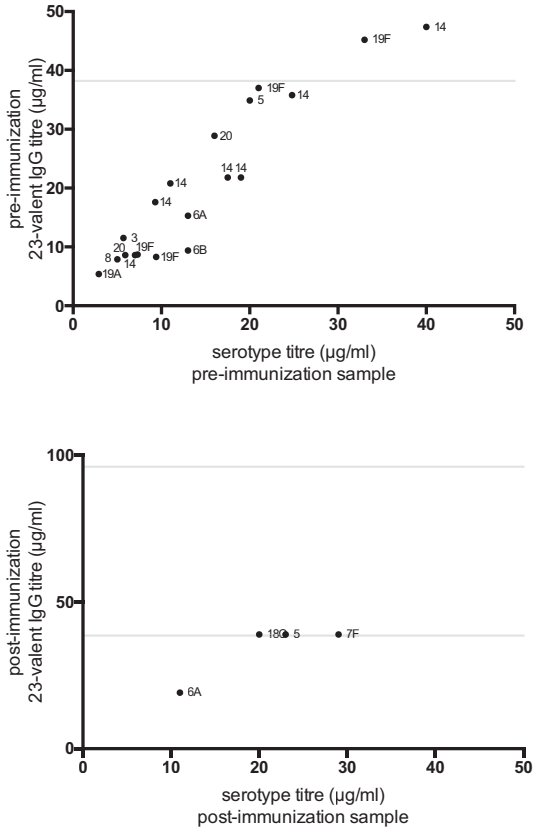
In conclusion, this is the first study evaluating the application of the 23-valent IgG VaccZyme™ anti-PCP IgG ELISA Kit for predicting *good* responders to PnPS-vaccination in a general hospital patient population setting. We showed that this assay can be a first screening

test in Pn-C vaccine-naïve patients to determine which patients in a general hospital setting do *not* need serotype-specific testing. This can reduce the number of PnC vaccine-naive patients needing PnPS serotyping in the low pre-test probability setting of a general hospital, thus lowering the threshold for testing for suspected PAD while simultaneously reducing overall costs.

REFERENCES

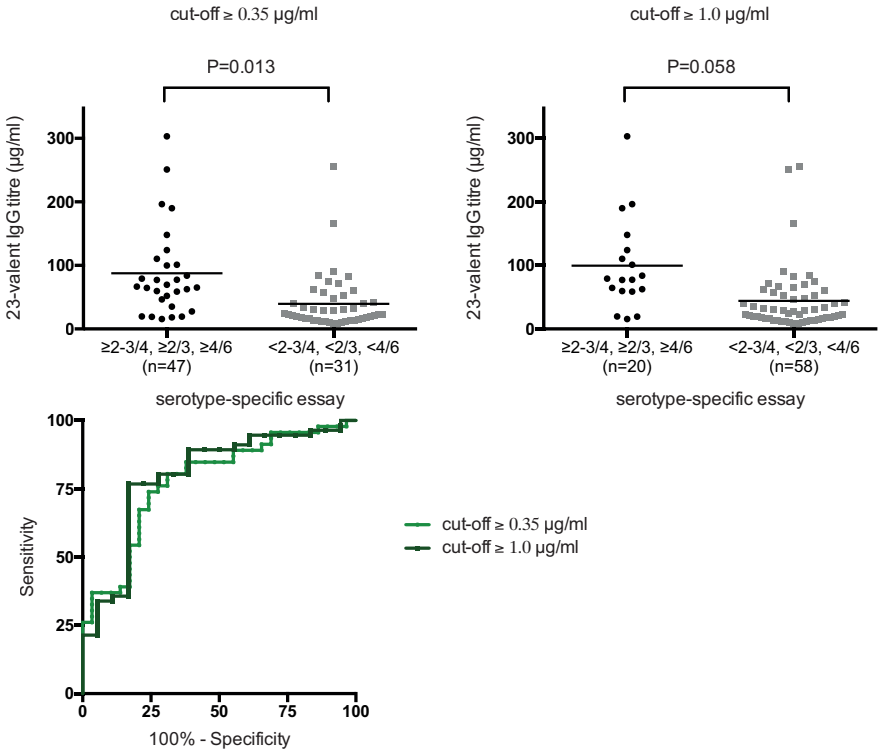
1. Orange JS, Ballow M, Stiehm ER, Ballas ZK, Chinen J, De La Morena M, et al. Use and interpretation of diagnostic vaccination in primary immunodeficiency: a working group report of the Basic and Clinical Immunology Interest Section of the American Academy of Allergy, Asthma & Immunology. J Allergy Clin Immunol. 2012 Sep;130(3 Suppl):S1-24.
2. Perez E, Bonilla FA, Orange JS, Ballow M. Corrigendum: Specific Antibody Deficiency: Controversies in Diagnosis and Management. Vol. 9, Frontiers in immunology. Switzerland; 2018. p. 450.
3. Perez E, Bonilla FA, Orange JS, Ballow M. Specific Antibody Deficiency: Controversies in Diagnosis and Management. Front Immunol. 2017;8:586.
4. Bonilla FA, Khan DA, Ballas ZK, Chinen J, Frank MM, Hsu JT, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. J Allergy Clin Immunol. 2015 Nov;136(5):1178–86.
5. de Vries E. Patient-centred screening for primary immunodeficiency, a multi-stage diagnostic protocol designed for non-immunologists: 2011 update. Clin Exp Immunol. 2012 Jan;167(1):108–19.
6. Daly TM, Hill HR. Use and clinical interpretation of pneumococcal antibody measurements in the evaluation of humoral immune function. Clin Vaccine Immunol. 2015 Feb;22(2):148–52.
7. Borgers H, Moens L, Picard C, Jeurissen A, Raes M, Sauer K, et al. Laboratory diagnosis of specific antibody deficiency to pneumococcal capsular polysaccharide antigens by multiplexed bead assay. Clin Immunol. 2010 Feb;134(2):198–205.
8. Balloch A, Licciardi P V, Tang MLK. Serotype-specific anti-pneumococcal IgG and immune competence: critical differences in interpretation criteria when different methods are used. J Clin Immunol. 2013 Feb;33(2):335–41.
9. Hare ND, Smith BJ, Ballas ZK. Antibody response to pneumococcal vaccination as a function of preimmunization titer. J Allergy Clin Immunol. 2009 Jan;123(1):195–200.
10. Balmer P, Cant AJ, Borrow R. Anti-pneumococcal antibody titre measurement: what useful information does it yield? J Clin Pathol. 2007 Apr;60(4):345–50.
11. Lopez B, Bahuaud M, Fieschi C, Mehlal S, Jeljeli M, Rogeau S, et al. Value of the Overall Pneumococcal Polysaccharide Response in the Diagnosis of Primary Humoral Immunodeficiencies. Front Immunol. 2017;8:1862.
12. Janssen WJM, Bloem AC, Vellekoop P, Driessen GJ, Boes M, van Montfrans JM. Measurement of pneumococcal polysaccharide vaccine responses for immunodeficiency diagnostics: combined IgG responses compared to serotype specific IgG responses. Vol. 34, Journal of clinical immunology. Netherlands; 2014. p. 3–6.
13. Schauer U, Stemberg F, Rieger CHL, Buttner W, Borte M, Schubert S, et al. Levels of antibodies specific to tetanus toxoid, Haemophilus influenzae type b, and pneumococcal capsular polysaccharide in healthy children and adults. Clin Diagn Lab Immunol. 2003 Mar;10(2):202–7.
14. Janssen LMA, Bassett P, Macken T, van Esch J, Pruijt H, Knoops A, et al. Mild Hypogammaglobulinemia Can Be a Serious Condition. Front Immunol. 2018;9:2384.
15. Pickering JW, Martins TB, Greer RW, Schroder MC, Astill ME, Litwin CM, et al. A multiplexed fluorescent microsphere immunoassay for antibodies to pneumococcal capsular polysaccharides. Am J Clin Pathol. 2002 Apr;117(4):589–96.
16. Sanders LA, Rijkers GT, Kuis W, Tenbergen-Meekes AJ, de Graeff-Meeder BR, Hiemstra I, et al. Defective antipneumococcal polysaccharide antibody response in children with recurrent respiratory tract infections. J Allergy Clin Immunol. 1993 Jan;91(1 Pt 1):110–9.

17. Siber GR, Chang I, Baker S, Fernsten P, O'Brien KL, Santosham M, et al. Estimating the protective concentration of anti-pneumococcal capsular polysaccharide antibodies. Vaccine. 2007 May;25(19):3816–26.
18. Trs R. Recommendations to assure the quality, safety and efficacy of pneumococcal conjugate vaccines Replacement. World Health. 2009;(October).
19. Paris K, Sorensen RU. Assessment and clinical interpretation of polysaccharide antibody responses. Ann Allergy Asthma Immunol. 2007 Nov;99(5):462–4.
20. Meek, B, A. Roos, A.J. van Houte AMEC. Bepaling antistoffen tegen Pneumokokken Polysachariden - Luminex. 2014;
21. Richtlijn interpretatie van uitslagen van respons op pneumokokken vaccinaties. 2016;7556576.
22. The Binding Site Group Ltd. VaccZyme ™ Anti-PCP IgG Enzyme Immunoassay Kit For in-vitro diagnostic use Product code : MK012. 2014;(1):12–4.
23. Wernette CM, Frasch CE, Madore D, Carlone G, Goldblatt D, Plikaytis B, et al. Enzyme-linked immunosorbent assay for quantitation of human antibodies to pneumococcal polysaccharides. Clin Diagn Lab Immunol. 2003 Jul;10(4):514–9.
24. Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. J Chiropr Med. 2016 Jun;15(2):155–63.
25. Ltd TBSG. Could VaccZyme PCP IgG ELISA be a cost effective pre-screen to PCP serotyping?
26. Stead A, Douglas JG, Broadfoot CJ, Kaminski ER, Herriot R. Humoral immunity and bronchiectasis. Clin Exp Immunol. 2002 Nov;130(2):325–30.
27. Parker AR, Park MA, Harding S, Abraham RS. The total IgM, IgA and IgG antibody responses to pneumococcal polysaccharide vaccination (Pneumovax(R)23) in a healthy adult population and patients diagnosed with primary immunodeficiencies. Vaccine. 2019 Feb;37(10):1350–5.
28. Rose MA, Buess J, Ventur Y, Zielen S, Herrmann E, Schulze J, et al. Reference ranges and cutoff levels of pneumococcal antibody global serum assays (IgG and IgG2) and specific antibodies in healthy children and adults. Med Microbiol Immunol. 2013 Aug;202(4):285–94.
29. Chua I, Lagos M, Charalambous BM, Workman S, Chee R, Grimbacher B. Pathogen-specific IgG antibody levels in immunodeficient patients receiving immunoglobulin replacement do not provide additional benefit to therapeutic management over total serum IgG. Vol. 127, The Journal of allergy and clinical immunology. United States; 2011. p. 1410–1.
30. Rubins JB, Alter M, Loch J, Janoff EN. Determination of antibody responses of elderly adults to all 23 capsular polysaccharides after pneumococcal vaccination. Infect Immun. 1999 Nov;67(11):5979–84.
31. Lakshman R, Gennery AR, Arkwright PD, Flood T, Abinun M, Spickett G, et al. Assessing immune responses to pneumococcal vaccines. Vol. 88, Archives of disease in childhood. England; 2003. p. 648–9.
32. Guevara-Hoyer K, Gil C, Parker AR, Williams LJ, Orte C, Rodriguez de la Pena A, et al. Measurement of Typhim Vi IgG as a Diagnostic Tool to Determine Anti-polysaccharide Antibody Production Deficiency in Children. Front Immunol. 2019;10:654.
33. Zhang X, Simmerman K, Yen-Lieberman B, Daly TM. Impact of analytical variability on clinical interpretation of multiplex pneumococcal serology assays. Clin Vaccine Immunol. 2013 Jul;20(7):957–61.
34. Daly TM, Pickering JW, Zhang X, Prince HE, Hill HR. Multilaboratory assessment of threshold versus fold-change algorithms for minimizing analytical variability in multiplexed pneumococcal IgG measurements. Clin Vaccine Immunol. 2014 Jul;21(7):982–8.
35. Tan CY, Immermann FW, Sebastian S, Pride MW, Pavliakova D, Belanger KA, et al. Evaluation of a Validated Luminex-Based Multiplex Immunoassay for Measuring Immunoglobulin G Antibodies in Serum to Pneumococcal Capsular Polysaccharides. mSphere. 2018 Aug;3(4).



Supplementary Figure 8.1. Titres of serotypes that contributed ≥50% to the 23-valent IgG titre plotted against the 23-valent IgG titre in the same sample.

Supplementary Figure 8.1. Lines are drawn at the cut-off values as previously calculated (38.2 µg/ml for pre-immunization samples; 38.5 and 96.1 µg/ml for post-immunization samples).



Supplementary Figure 8.2. 23-valent pneumococcal IgG titres (µg/ml) in samples with and samples without ≥ 2-3/4, ≥ 2/3 or ≥ 4/6 serotypes above the cut-off values of 0.35 µg/ml (A) and 1.0 µg/ml (B).

Supplementary Figure 8.2. P-values were calculated in an unpaired T-test. Receiver operating characteristic (ROC) curves of sensitivity versus specificity of the 23-valent pneumococcal IgG titre using two different cut-off levels for the serotype-specific assay (C).

Chapter 9

The clinical relevance of IgM and IgA anti-pneumococcal polysaccharide ELISA assays in patients with suspected antibody deficiency

Lisanne MA Janssen, Michiel Heron, Jean-Luc Murk,
Alexander C.A.P. Leenders, Ger T. Rijkers, Esther de Vries

Clin Exp Immunol. 2021; 205(2):213-221

ABSTRACT

Unlike IgG pneumococcal polysaccharide(PnPS)-antibodies, PnPS IgA and IgM-antibodies are not routinely determined for the assessment of immunocompetence. It is not yet known whether an isolated inability to mount a normal IgM or IgA-PnPS response should be considered a relevant primary antibody deficiency (PAD). We studied the clinical relevance of anti-PnPS IgM and IgA-assays in patients with suspected primary immunodeficiency in a large teaching hospital in 's-Hertogenbosch, the Netherlands. Serotype-specific-PnPS IgG-assays were performed, subsequently, 23-valent-PnPS IgG-assays (anti-PnPS IgG-assays), and later anti-PnPS IgA and IgM-assays were performed in archived material (240 patients; 304 samples). 11/65 pre-immunisation and 6/10 post-immunisation samples from good responders to PnPS-serotype-specific IgG-testing, had decreased anti-PnPS IgA and/or IgM-titres. Of these, three pre-immunisation and no post-immunisation samples were from patients previously classified as 'no PAD'. Determination of anti-PnPS IgA and IgM in addition to anti-PnPS IgG did not reduce the need for serotype-specific-PnPS IgG-testing to assess immunocompetence (ROC-analysis of post-immunisation samples: anti-PnPS IgA+IgG AUC 0.80(95%-CI 0.63-0.97); anti-PnPS IgM+IgG AUC 0.80(95%-CI 0.62-0.98); anti-PnPS IgA+IgG+IgM AUC 0.71(95%-CI 0.51-0.91); anti-PnPS IgG AUC 0.93(95%-CI 0.85-1.00)). Our data show that patients, classified as having an intact antibody response based on measurement of serotype-specific-PnPS IgG, still can display impaired anti-PnPS IgM- and IgA-responses, and that the additional measurement of anti-PnPS IgA and IgM could not reduce the need for serotype-specific IgG-testing. Future studies are needed to investigate the clinical relevance of potential 'specific IgA- or IgM-antibody deficiency' in patients with recurrent airway infections in whom no PAD could be diagnosed according to the current definitions.

INTRODUCTION

Specific antibody deficiency (SPAD) is defined as the inability to mount an IgG antibody response to purified *Streptococcus pneumoniae* capsular polysaccharide antigens in the presence of normal immunoglobulin concentrations and normal antibody responses to protein antigens [1]. SPAD was first reported in a small group of patients in the early 1980s [2,3]. Patients with SPAD suffer from recurrent ear-nose-throat (ENT) and airway infections with encapsulated bacteria. Pneumococcal polysaccharide (PnPS) antibodies can be measured as the cumulative titre of antibodies to all 23 serotypes present in the PnPS vaccine (hereafter called "anti-PnPS IgG assay"), or as individual serotype-specific antibodies (hereafter called "serotype-specific PnPS IgG testing") [4–6]. Such serotype-specific PnPS IgG testing is expensive, not widely available, and interpretation of the results has proven to be challenging [7,8]. The anti-PnPS IgG assay has been shown to be a reliable screening test for poor [9] as well as for good [10] serotype-specific PnPS IgG responders to PnPS vaccine in conjugated pneumococcal (Pn-C) vaccine naïve patients. This reduces the number of patients needing serotype-specific PnPS IgG testing, thus reducing the costs while maintaining the quality of the diagnostic assessment for potential SPAD.

The cumulative PnPS antibody response can also be measured for IgM and IgA type antibodies, but this is not routinely performed for the assessment of immunocompetence or risk of pneumococcal infection [11–14]. Anti-PnPS IgA and IgM antibody responses have been investigated in healthy donors [12–14], patients with common variable immunodeficiency disorders (CVID) [11,15], patients with primary antibody deficiency (PAD) [16], and children with transient hypogammaglobulinemia of infancy (THI) [17]. The anti-PnPS IgA and IgM assays identify CVID patients with greater risk of infectious and non-infectious (autoimmunity, enteropathy) complications [11,15,16,18] and predict the disease course in young children diagnosed with antibody deficiency [19]. However, it is unknown whether an isolated inability to mount a normal IgM or IgA PnPS response should be considered a clinically relevant PAD. Theoretically, such specific IgM or IgA antibody deficiencies could be clinically relevant, because IgM and IgA are predominant immunoglobulin isotypes in the upper and lower airways with different effector mechanisms than IgG [20,21].

In this study, we investigated the clinical relevance of anti-PnPS IgM and IgA assays in addition to the anti-PnPS IgG assay, when analysing patients for potential PAD in a general hospital population. Our first objective was to investigate whether there were patients in our cohort with recurrent ENT and/or respiratory tract infections labelled as 'no PAD' based on a good response in serotype-specific PnPS IgG assays [10] with a reduced anti-PnPS IgA and/or IgM response. Second, we investigated whether adding anti-PnPS IgA and/or IgM assays to the anti-PnPS IgG assay could reduce the need for serotype-specific PnPS testing.

MATERIALS AND METHOD

Study design

Anti-PnPS IgA and IgM assays were performed on 304 blood samples, obtained from 240 patients in regular patient care who were analysed for the potential presence of primary immunodeficiency (PID) in the Jeroen Bosch Hospital (JBZ) in 's-Hertogenbosch, the Netherlands, between February 2012 and December 2018. Of these, 61 samples were from 49 patients who were previously vaccinated with the Pn-C vaccine. Residual samples were stored at ≤ -80 °C and later retrieved from the laboratory to perform anti-PnPS IgA and IgM assays between September and November 2019; anti-PnPS IgG assays were previously performed (and published) between August and September 2018 [10]. Most patients (n=84) were diagnosed with unclassified primary antibody deficiency (unPAD): deficiency of IgG, and/or combination(s) of deficiency of IgG-subclass(es), IgM, IgA or specific antibodies. Thirteen patients were diagnosed with common variable immunodeficiency disorders (CVID), four with selective IgA deficiency (sIgAdef), three with selective IgM deficiency (sIgMdef), two with transient hypogammaglobulinemia of infancy (THI), four with another type of PID than PAD, one with human immunodeficiency virus infection (HIV), eight with secondary immunodeficiency. In 87 patients it was concluded that they did not have a PID; in 34 patients there was no definitive diagnosis because of incomplete data. High-resolution CT (HRCT) scans were available for 68 patients; these were scored by a thoracic radiologist according to the "Chest CT in ADS" criteria [23]. The study was granted ethical approval by the local medical ethics committee and written informed consent was obtained from all adults and parents of the children.

Methods

ELISA for the quantification of anti-PnPS IgG, IgM, and IgA

Commercially available ELISA kits (VaccZyme™ pneumococcal capsular polysaccharide ELISAs, The Binding Site Group Limited, UK) were used to measure anti-PnPS IgG, IgM and IgA according to the manufacturer's instructions. Absorption of interfering anti-cell wall polysaccharide (anti-CWPS) antibodies was incorporated in these assays. Cut-offs used for Pn-C vaccine-naïve patients were the lower limit of the normal range (LLNR) as determined by Parker et al. in healthy adults (pre-immunisation: anti-PnPS IgG 10 µg/ml, anti-PnPS IgA 6 U/ml, anti-PnPS IgM 16 U/ml; post-immunisation: anti-PnPS IgG 77 µg/ml, anti-PnPS IgA 78 U/ml, anti-PnPS IgM 60 U/ml) [13].

Quantification of serotype-specific anti-PnPS IgG antibodies

The Luminex multiplex immunoassay was used to measure serotype-specific IgG antibodies against PnPS as previously described [10], including CPS 22F adsorption to block anti-CWPS antibodies [4]. For assessing the response to PnPS vaccination a blood sample was drawn 4-8

weeks after intramuscular vaccination with one dose of 23-valent PnPS vaccine (Pneumovax 23; Merck, Sharp & Dohme BV, Haarlem, The Netherlands). A good response to PnPS vaccination was defined according to the international consensus response criteria [24].

Statistical Analysis

Data were analysed using SPSS 27.0 software (SPSS Inc., Chicago, IL, USA) and Graphpad Prism 6.0 software (GraphPad Software, La Jolla, CA, USA) for Mac. The Mann-Whitney *U* test was used for unpaired comparisons of anti-PnPS IgG, IgA, and IgM titres in: 1) pre- and post-immunisation samples (often both were not available from the same patient), 2) poor and good responders to PnPS vaccination as determined by the serotype-specific assay, 3) patients with and without PAD, and 4) patients with and without bronchiectasis. Separate analyses were performed for patients who were previously immunised with Pn-C vaccine. Spearman correlation coefficient (*r*) was estimated to determine the linear association between the anti-PnPS IgG, IgM or IgA titres and serum immunoglobulins. The results were interpreted according to the degree of association as strong (r = 0.7-1), moderate (r = 0.5-0.7), or low (r = 0.3-0.5) after taking significant correlation values ($p < 0.05$) into consideration. In order to be able to compare anti-PnPS IgA and IgM titres (U/ml) with anti-PnPS IgG titres (µg/ml), these values in our dataset were standardised by converting them into z-scores. To determine whether the sum of anti-PnPS IgG, IgA and/or IgM titres could better predict whether that patient was a good or a poor responder to PnPS vaccination as assessed by the serotype-specific assay, compared to the anti-PnPS IgG titre alone, receiver operating characteristics (ROC) curves were plotted and the areas under the curves (AUCs) were calculated. This was done separately for pre- and 4-8 weeks post-immunisation titres. All tests were two-tailed and *p*-values <0.05 were considered to be statistically significant.

RESULTS

The baseline characteristics of the 304 blood samples, obtained from 240 patients, are summarised in **Table 9.1**. The age-specific responses to PnPS vaccination are shown in **Supplementary Figure 9.1** for vaccine-naïve patients and in **Supplementary Figure 9.2** for pre-immunisation titres of Pn-C pre-vaccinated patients. In vaccine-naïve patients, the anti-PnPS IgM titres pre-immunisation and >8 weeks post-immunisation were lower in patients aged 61-80 years, compared to patients aged 0-20 years (p = 0.0001 and p = 0.0002, respectively). Age did not influence the anti-PnPS IgA or IgG response in this vaccine-naïve patient cohort with suspected PID. In Pn-C pre-vaccinated patients with suspected PID there was no significant difference between the pre-immunisation anti-PnPS IgM, IgA and IgG titres at 1-2 years of age and ≥8 years of age (p = 0.546, p = 0.497 and p = 0.999, respectively). Because of too few data of post-immunisation titres in Pn-C-pre-vaccinated patients, this analysis could not be done for post-immunisation titres in this group.

Comparison of all cumulative antibody tests in all samples

Spearman's correlation analysis revealed a moderate correlation between anti-PnPS IgG and anti-PnPS IgA (r = 0.52, p < 0.0001), while a poor correlation was observed between anti-PnPS IgM and anti-PnPS IgA (r = 0.39, p < 0.0001) and anti-PnPS IgG and anti-PnPS IgM (r = 0.23, p < 0.0001; **Supplementary Figure 9.3**). There was a moderate correlation between anti-PnPS IgM and the serum IgM level (r = 0.54, p < 0.0001; **Supplementary Figure 9.4**). Poor correlations were found between anti-PnPS IgA and serum IgA (r = 0.38, p < 0.0001) and anti-PnPS IgG and serum IgG (r = 0.03, p = 0.665). As expected, IgA and IgM deficient patients did not produce anti-PnPS IgA or IgM, respectively.

Patients previously classified as no-PAD based on their IgG response only

To investigate whether patients from our cohort with recurrent airway infections who had been classified as 'no PAD' based on normal serotype-specific PnPS IgG vaccination response and normal serum immunoglobulin levels, could have defective anti-PnPS IgA and/or IgM responses, pre- and post-immunisation anti-PnPS IgA and IgM titres were divided into four groups (IgA/IgM both decreased, only IgA decreased, only IgM decreased and IgA/IgM both normal; **Figure 9.1A and B**). Eleven of 65 pre-immunisation samples and 6 of 10 post-immunisation samples from patients with a good response to PnPS serotype-specific IgG testing, had decreased anti-PnPS IgA and/or IgM titres. Of these, three pre-immunisation samples and none of the post-immunisation samples were from patients who were previously classified as 'no PAD' (**Figure 9.2**). The data therefore indicate that up to 60% (6/10) of patients with an adequate anti-PnPS IgG response, still can display defects in the ability to generate a sufficient anti-PnPS IgM and/or IgA response.

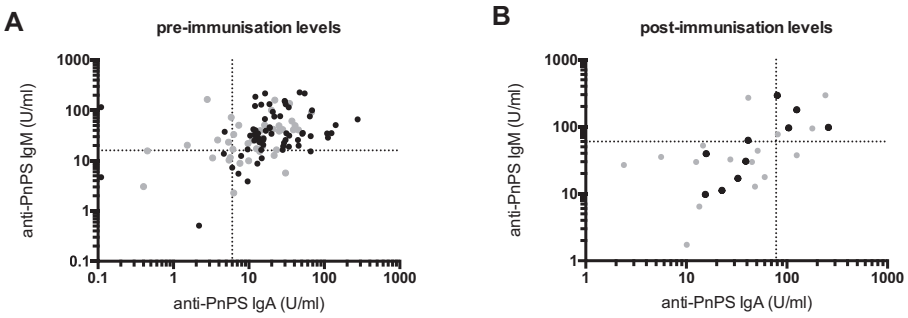


Figure 9.1. Pre- (A) and post-immunisation (B) anti-PnPS IgA and IgM titres distinguished four immunological groups: 1) IgA/IgM both decreased, 2) only IgA decreased, 3) only IgM decreased and 4) IgA/IgM both normal.

Figure 9.1. LLNR cut-offs: 6 U/ml for pre- and 78 U/ml for post-immunisation anti-PnPS IgA; 15 U/ml for pre- and 60 U/ml for post-immunisation anti-PnPS IgM, according to Parker et al. [13]. Poor serotype-specific PnPS IgG responders are coloured grey; good serotype-specific PnPS IgG responders are coloured black. In figure A, the anti-PnPS IgA values of 2 samples have been rounded from 0.0 to 0.1 U/ml, to make these points visible in the logarithmic scale of the figure.

The added value of anti-PnPS IgA and IgM assays in Pn-C vaccine-naïve patients

The anti-PnPS IgG, IgA and IgM concentrations pre-immunisation and in response to PnPS vaccination in all Pn-C vaccine-naïve patients are shown in **Figure 9.3**, categorised as either good or poor responders as assessed by the serotype-specific IgG assay in the same samples. In good and poor IgG responders, the concentration increase from pre- to 4-8 weeks post-immunisation was significant for anti-PnPS IgG and IgA, but not for anti-PnPS IgM. Even when outliers were omitted (open circles in **Figure 9.3**), the anti-PnPS IgA and IgG response remained significant in poor IgG responders (anti-PnPS IgA: 13.3 vs. 27.4 U/ml; $p = 0.05$ | anti-PnPS IgG: 12.7 vs. 21.1 µg/ml; $p = 0.02$). Also, in patients of whom both pre- and post-immunisation samples were available, the concentration increase from pre- to 4-8 weeks post-immunisation was significant for anti-PnPS IgG and IgA, but not for anti-PnPS IgM (**Supplementary Figure 9.5**). Only the anti-PnPS IgG fold increase could reliably discriminate between poor- or good responders to serotype-specific PnPS IgG vaccination [ROC analysis: AUC 0.90 (95% CI 0.76-1.00)], while the anti-PnPS IgM and -IgA could not [ROC analysis; anti-PnPS IgM: AUC 0.59 (95% CI 0.33-0.85) and anti-PnPS IgA: AUC 0.75 (95% CI 0.50-1.00); **Supplementary Figure 9.6**].

Table 9.1. Baseline characteristics.

	Pn-C pre-vaccinated patients	
	pre-immunisation	4-8 weeks post-immunisation
Number of samples	54	2
Age at time of measurement (years, median, IQR range)	4.7 (2.7-6.1)	*
Gender (% female)	31%	#

*Table 9.1. * Statistical analysis not possible because of too few samples (n=2). These patients were 6.7 and 3.9 years old at the time of measurement; #See under '*'; statistical analysis not possible because of too few samples (n=2). These patients were both girls.*

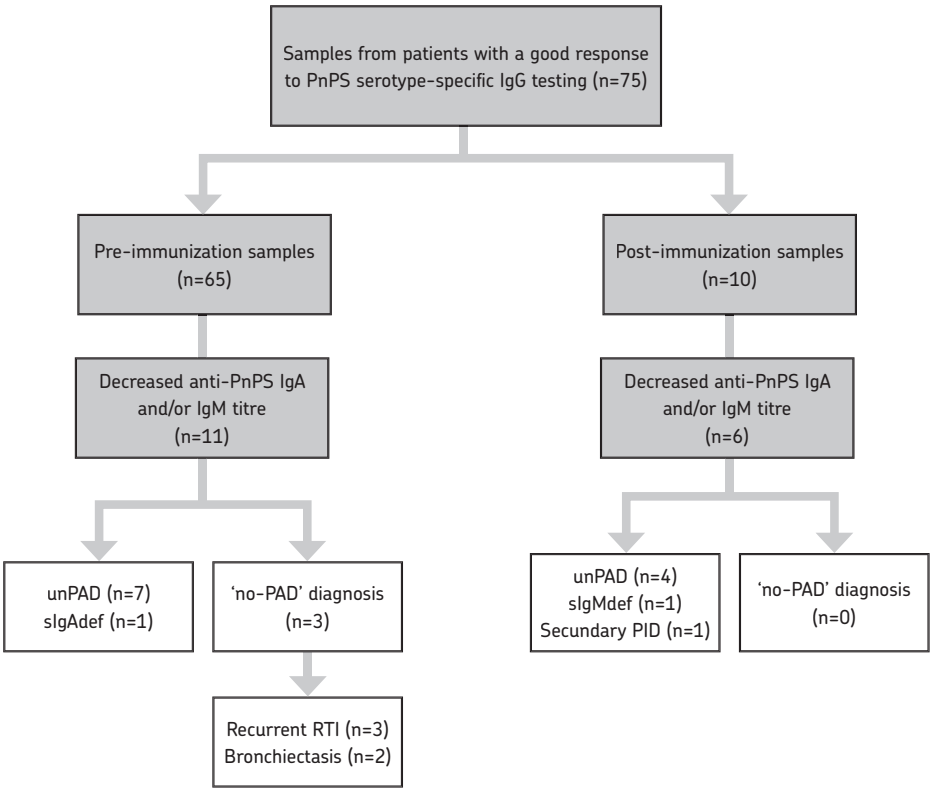


Figure 9.2. Patients previously classified as 'no PAD' based on their IgG response, with abnormal results in the anti-PnPS IgA and/or IgM assays.

Figure 9.2. Abbreviations: PAD, primary antibody deficiency; PID, primary immunodeficiency; PnPS, pneumococcal polysaccharide; RTI, respiratory tract infections; sIgAdef, selective IgA deficiency; sIgMdef, selective IgM deficiency; unPAD, unclassified primary antibody deficiency.

	Vaccine naïve patients		
>8 weeks post-immunisation	pre-immunisation	4-8 weeks post-immunisation	>8 weeks post-immunisation
5	175	26	42
4.6 (4.5-6.8)	42.2 (21.8-61.2)	49.0 (36.5-65.8)	49.4 (16.2-68.0)
20%	64%	62%	45%

Abbreviations: IQR, interquartile range; Pn-C, pneumococcal conjugated.

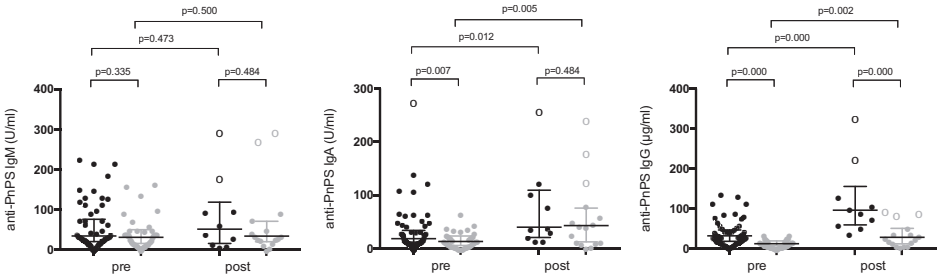


Figure 9.3. Pre- and post-immunisation cumulative anti-PnPS IgM, IgA, and IgG titres for good (black dots) and poor (grey dots; outliers marked as open circles) responders as assessed by serotype-specific PnPS IgG testing.

Figure 9.3. P-values were calculated with Mann-Whitney U tests.

Next, we evaluated whether adding anti-PnPS IgM and/or IgA assays could reduce the requirement for serotype-specific analyses, as compared to conducting only the anti-PnPS IgG assay. The sum of the z-scores of anti-PnPS IgA and IgG, anti-PnPS IgM and IgG, and anti-PnPS IgA, IgG and IgM were separately compared for pre- and 4-8 weeks post-immunisation titres with the serotype-specific PnPS IgG vaccination response. The results of the ROC curve analyses are shown in **Figure 9.4A-C**. The sum of the z-scores of post-immunisation anti-PnPS IgA + IgG and anti-PnPS IgM + IgG could best discriminate between good and poor responders as determined by the serotype-specific PnPS IgG vaccination response [ROC analysis; AUC 0.80 (95% CI 0.63-0.97) and 0.80 (95% CI 0.62-0.98), respectively]. However, the discriminative power of using the anti-PnPS IgG assay alone was higher [ROC analysis; pre-immunisation: AUC 0.84 (95% CI 0.76-0.91) and post-immunisation: AUC 0.93 (95% CI 0.85-1.00) [10]].

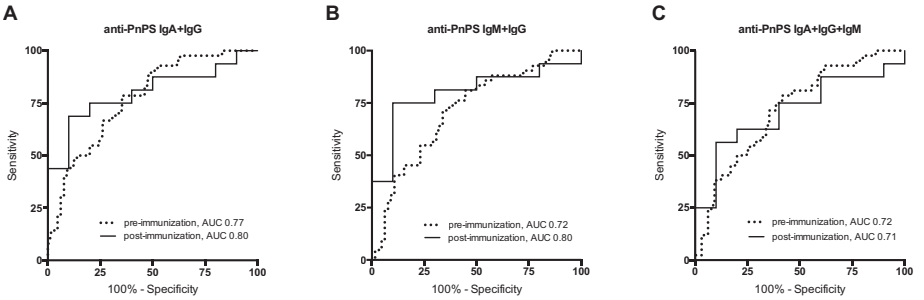


Figure 9.4. Receiver operating characteristic (ROC) curves.

Figure 9.4. Receiver operating characteristic (ROC) curves of sensitivity vs. specificity for the sum of z-scores of pre- and 4-8 weeks post-immunisation pneumococcal immunoglobulins vs. serotype-specific IgG response to vaccination: (A) anti-PnPS IgG + IgA z-scores, (B) anti-PnPS IgG + IgM z-scores, (C) anti-PnPS IgG + IgA + IgM z-scores.

Comparison of patients with and without PAD

Pre-immunisation anti-PnPS IgG, IgA, and IgM titres were significantly lower in Pn-C vaccine naïve patients with PAD, compared to those without PAD (**Supplementary Table 9.1A**). This comparison could not be made for post-immunisation anti-PnPS IgG, IgA, and IgM titres, because only one patient did not have PAD. Pn-C vaccine naïve patients with PAD had significantly more often pre-immunisation anti-PnPS IgG and IgM titres below the LLNR, compared to patients without PAD (**Supplementary Table 9.1B**). In addition, a number of PAD patients had post-immunisation anti-PnPS IgG (16/23, 70%), IgA (18/23, 78%) and IgM (15/23, 65%) titres below the LLNR. In Pn-C pre-vaccinated patients anti-PnPS IgG, IgA, and IgM titres were not statistically different between patients with and without PAD (**Supplementary Table 9.1C**). This comparison could not be made for post-immunisation titres, because there were only two post-immunisation samples in the Pn-C pre-vaccinated patient group.

Comparison of Pn-C vaccine naïve patients with and without bronchiectasis

The prevalence of bronchiectasis was identical in patients with post-immunisation anti-PnPS IgA or IgM titres above and below the LLNR (75% in all categories). Also, both pre- and post-immunisation IgA and IgM titres were not lower in patients with bronchiectasis, compared to those without bronchiectasis (**Supplementary Table 9.2A and B**).

Comparison of Pn-C pre-vaccinated with Pn-C vaccine naïve patients

Pn-C pre-vaccinated patients had significantly higher pre-immunisation anti-PnPS IgM titres (median 56 U/ml, range 8-270 U/ml), compared to Pn-C vaccine naïve patients (median 35 U/ml, range 1-305 U/ml, $p = 0.001$). Anti-PnPS IgG and IgA pre-immunisation titres were not significantly different between Pn-C pre-vaccinated and PnC-vaccine naïve patients (**Supplementary Table 9.3**).

DISCUSSION

Principal findings

In this study we have expanded our analysis of PnPS antibody levels and response to vaccination by including, next to IgG, also IgM and IgA anti-PnPS antibodies. Our data show that patients, classified as having an intact antibody response based on measurement of serotype-specific PnPS IgG, still can display impaired anti-PnPS IgM and IgA responses. Isolated decreased anti-PnPS IgM and in particular anti-PnPS IgA response might have clinical relevance. Decreased anti-PnPS IgA and IgM responses have been reported in healthy adult blood donors [12,13], but have also been associated with a greater rate of respiratory infections in patients with CVID [11,15] and PAD [16]. In patients with recurrent ENT or airway infections in whom no PAD could be diagnosed according to the current standards, the clinical relevance of isolated decreased anti-PnPS IgA and/or IgM responses has not yet been investigated. In this study we measured anti-PnPS IgA and IgM levels in order to determine whether - in addition to 'specific IgG antibody deficiency' - 'specific IgA or IgM antibody deficiency' might be a clinically relevant form of antibody deficiency. None of the patients with a decreased anti-PnPS IgM or IgA response had been classified as 'no PAD' based on serotype-specific PnPS IgG testing and serum immunoglobulin levels. Therefore, we could not determine its clinical relevance based on our data.

To gain further insight in the clinical relevance of anti-PnPS IgA and IgM assays, we investigated whether adding these assays to the anti-PnPS IgG assay could reduce the need for the more expensive and difficult to interpret serotype-specific PnPS IgG testing [10]. ROC analysis showed that the discriminative power of the anti-PnPS IgG assay alone to detect good responders was superior to any other combination. Therefore, based on our data, it does not seem useful for a clinician in a general hospital to request anti-PnPS IgA and IgM assays in addition to anti-PnPS IgG assay in order to reduce the need for serotype-specific PnPS IgG testing.

Comparison with existing literature

Previous studies have reported conflicting results concerning the correlation between PnPS responses for all three immunoglobulin isotypes with their respective serum levels. Similar to our results, poor correlations were found in a healthy population by Parker et al. [13] and a PAD cohort by De Carlos et al. [16]. In contrast, Cavaliere et al. did find a significant correlation in a CVID cohort [11]. By definition CVID patients have decreased IgM and/or IgA concentrations, and a significant proportion would have decreased anti-PnPS IgM and IgA responses. This might explain the good correlation between PnPS responses and their respective serum immunoglobulins in CVID patients, which is not expected in 'milder' PAD patients or a healthy population.

In contrast to Cavaliere et al. we did not find a higher bronchiectasis prevalence in patients with impaired anti-PnPS IgA and IgM responses [11]. However, while our cohort included patients with unPAD and milder forms of CVID with an 'infection-only' phenotype, Cavaliere et al. mainly included severely affected CVID patients with immune dysregulation complications. Also, our results might be biased towards a higher bronchiectasis prevalence, because HRCT scans were only performed in patients in whom pathology was expected.

Previous studies have reported on the influence of age on the anti-PnPS IgM and IgA response in healthy adults and highlighted the importance of age-specific reference ranges. Park, Parker and Ademokun et al. reported that older adults (>60 years) had lower anti-PnPS IgM and IgA responses compared to younger adults [13,22,26]. We did find that with increasing age the pre-immunisation titres of anti-PnPS IgM antibodies were lower, but in our cohort of patients with suspected PID we did not find lower anti-PnPS IgM and IgA responses with increasing age. Our results, however, represent a mixture of patients with and without PAD with both normal and impaired anti-PnPS IgG, IgA and IgM responses. Future studies in large healthy adult populations are needed to improve the evidence on age-specific reference ranges for pre- and post-immunisation anti-PnPS IgM and IgA titres.

The higher pre-immunisation anti-PnPS IgM titres in the PCV primed paediatric group, compared to the unprimed adult group can be due to lower age and the different immunogenicity of PCV. Contrary to the unconjugated PnPS vaccine, PCV induces a T-dependent, more pronounced memory response. A single dose of PCV is able to induce a significant IgM response measurable 1 month after vaccination [27]. It would be interesting to investigate this issue in prospective cohort studies comparing PCV primed and unprimed groups.

Limitations

Our study has several limitations. First, the timepoint to take post-immunisation samples 4-6 weeks after vaccination may be adequate for IgG and IgA antibodies, but IgM antibodies could already have been declining. This could explain our finding that, in vaccine-naïve patients, good serotype-specific PnPS IgG responders showed a significant anti-PnPS IgA and IgG rise, but not a significant anti-PnPS IgM rise. Parker et al. also reported a high percentage of healthy individuals with decreased anti-PnPS IgM concentrations 4-6 weeks post-vaccination [13]. In contrast, Schütz et al. found that in healthy adults anti-PnPS IgM titres reached its maximum 3-4 weeks post-immunization, and remained at a plateau for 3 months [14].

Second, anti-PnPS IgA and IgM were only measured in blood, not in mucosal tissues or secretions. While the pneumococcal conjugate vaccine (PCV) and PnPS vaccine after priming with PCV have been shown to be able to induce protective mucosal IgA antibodies [28,29], it is unknown whether this also occurs after immunisation with PnPS vaccine alone. Most infectious pathogens enter the host via mucosal surfaces, where mucosal IgA

represent the hallmark of immune responses [30]. In future studies it would be interesting to investigate anti-PnPS IgA responses in both blood and mucosal tissues to learn more about the clinical relevance of a defective anti-PnPS IgA response in the circulation.

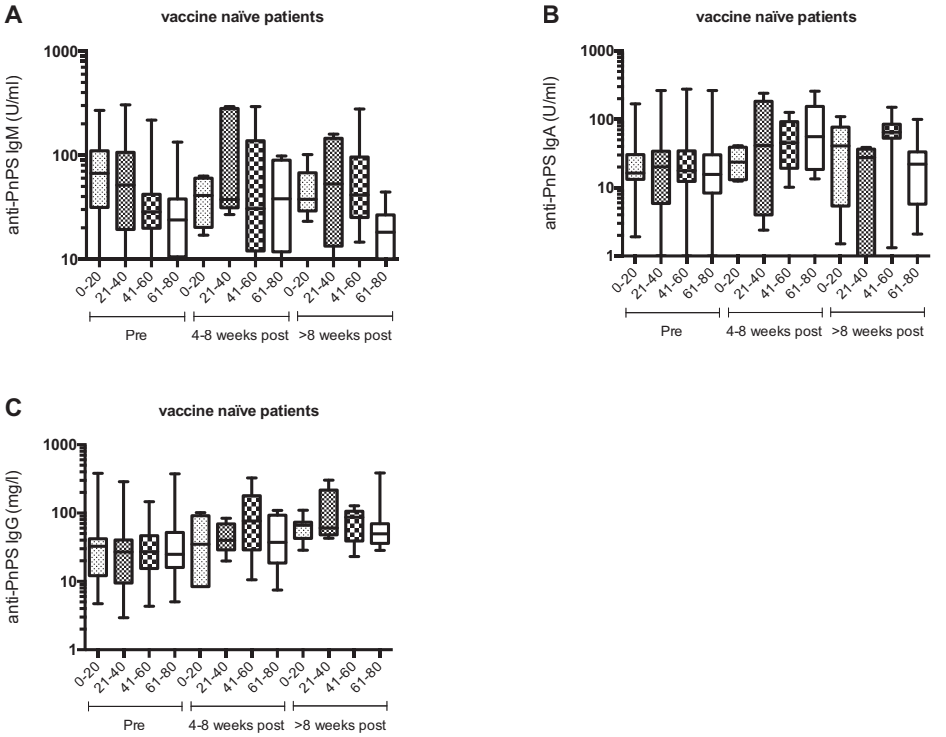
Conclusion and implication for future research

Our study shows that patients classified as having an intact PnPS antibody response based on measurement of IgG antibodies, still can display defective anti-PnPS IgA and IgM responses. In addition, we show that the additional measurement of anti-PnPS IgA and -IgM could not reduce the need for serotype-specific PnPS IgG testing. However, our sample size was too small to draw any definitive conclusions on the clinical relevance of our findings. Future studies are needed in patients with recurrent ENT or airway infections in whom no PAD could be diagnosed according to the current standards, to investigate whether – in addition to 'specific IgG antibody deficiency' - also 'specific IgA or IgM antibody deficiency' can be a clinically relevant form of antibody deficiency.

REFERENCES

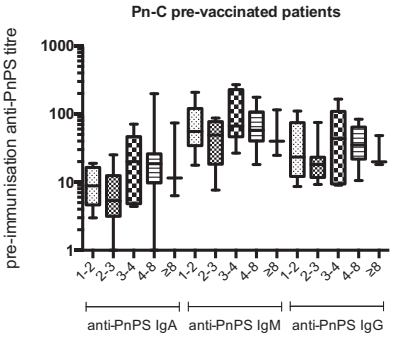
1. Sorensen RU, Edgar D. Specific Antibody Deficiencies in Clinical Practice. J allergy Clin Immunol Pract. 2019 Mar;7(3):801–8.
2. Saxon A, Kobayashi RH, Stevens RH, Singer AD, Stiehm ER, Siegel SC. In vitro analysis of humoral immunity in antibody deficiency with normal immunoglobulins. Clin Immunol Immunopathol. 1980 Oct;17(2):235–44.
3. French MA, Harrison G. Systemic antibody deficiency in patients without serum immunoglobulin deficiency or with selective IgA deficiency. Clin Exp Immunol. 1984 Apr;56(1):18–22.
4. Pickering JW, Martins TB, Greer RW, Schroder MC, Astill ME, Litwin CM, et al. A multiplexed fluorescent microsphere immunoassay for antibodies to pneumococcal capsular polysaccharides. Am J Clin Pathol. 2002 Apr;117(4):589–96.
5. Biagini RE, Schlottmann SA, Sammons DL, Smith JP, Snawder JC, Striley CAF, et al. Method for simultaneous measurement of antibodies to 23 pneumococcal capsular polysaccharides. Clin Diagn Lab Immunol. 2003 Sep;10(5):744–50.
6. Marchese RD, Jain NT, Antonello J, Mallette L, Butterfield-Gerson KL, Raab J, et al. Enzyme-linked immunosorbent assay for measuring antibodies to pneumococcal polysaccharides for the PNEUMOVAX 23 vaccine: assay operating characteristics and correlation to the WHO international assay. Clin Vaccine Immunol. 2006 Aug;13(8):905–12.
7. Daly TM, Hill HR. Use and clinical interpretation of pneumococcal antibody measurements in the evaluation of humoral immune function. Clin Vaccine Immunol. 2015 Feb;22(2):148–52.
8. Balloch A, Licciardi P V, Tang MLK. Serotype-specific anti-pneumococcal IgG and immune competence: critical differences in interpretation criteria when different methods are used. J Clin Immunol. 2013 Feb;33(2):335–41.
9. Lopez B, Bahuaud M, Fieschi C, Mehlal S, Jeljeli M, Rogeau S, et al. Value of the Overall Pneumococcal Polysaccharide Response in the Diagnosis of Primary Humoral Immunodeficiencies. Front Immunol. 2017;8:1862.
10. Janssen LMA, Heron M, Murk J-L, Leenders ACAP, Rijkers GT, de Vries E. Focusing on Good Responders to Pneumococcal Polysaccharide Vaccination in General Hospital Patients Suspected for Immunodeficiency. A Decision Tree Based on the 23-Valent Pneumococcal IgG Assay. Front Immunol. 2019;10:2496.
11. Cavaliere FM, Milito C, Martini H, Schlesier M, Dräger R, Schütz K, et al. Quantification of IgM and IgA anti-pneumococcal capsular polysaccharides by a new ELISA assay: a valuable diagnostic and prognostic tool for common variable immunodeficiency. J Clin Immunol. 2013 May;33(4):838–46.
12. Parker AR, Allen S, Harding S. Concentration of anti-pneumococcal capsular polysaccharide IgM, IgG and IgA specific antibodies in adult blood donors. Pract Lab Med. 2016 Aug;5:1–5.
13. Parker AR, Park MA, Harding S, Abraham RS. The total IgM, IgA and IgG antibody responses to pneumococcal polysaccharide vaccination (Pneumovax(R)23) in a healthy adult population and patients diagnosed with primary immunodeficiencies. Vaccine. 2019 Feb;37(10):1350–5.
14. Schütz K, Hughes RG, Parker A, Quinti I, Thon V, Cavaliere M, et al. Kinetics of IgM and IgA antibody response to 23-valent pneumococcal polysaccharide vaccination in healthy subjects. J Clin Immunol. 2013 Jan;33(1):288–96.
15. Pulvirenti F, Milito C, Cavaliere FM, Mezzaroma I, Cinetto F, Quinti I. IGA Antibody Induced by Immunization With Pneumococcal Polysaccharides Is a Prognostic Tool in Common Variable Immune Deficiencies. Front Immunol. 2020;11:1283.

16. Echeverría de Carlos A, Gómez de la Torre R, García Carus E, Caminal Montero L, Bernardino Díaz López J, Suárez Casado H, et al. Concentrations of Pneumococcal IgA and IgM are compromised in some individuals with antibody deficiencies. J Immunoassay Immunochem. 2017;38(5):505–13.
17. Moschese V, Cavaliere FM, Graziani S, Bilotta C, Milito C, Chini L, et al. Decreased IgM, IgA, and IgG response to pneumococcal vaccine in children with transient hypogammaglobulinemia of infancy. Vol. 137, The Journal of allergy and clinical immunology. United States; 2016. p. 617–9.
18. Carsetti R, Rosado MM, Donnanno S, Guazzi V, Soresina A, Meini A, et al. The loss of IgM memory B cells correlates with clinical disease in common variable immunodeficiency. J Allergy Clin Immunol. 2005 Feb;115(2):412–7.
19. Janssen WJM, Nierkens S, Sanders EA, Boes M, van Montfrans JM. Antigen-specific IgA titres after 23-valent pneumococcal vaccine indicate transient antibody deficiency disease in children. Vaccine. 2015 Nov;33(46):6320–6.
20. Woof JM, Kerr MA. The function of immunoglobulin A in immunity. J Pathol. 2006 Jan;208(2):270–82.
21. Brandtzaeg P. Induction of secretory immunity and memory at mucosal surfaces. Vaccine. 2007 Jul;25(30):5467–84.
22. Park S, Nahm MH. Older adults have a low capacity to opsonize pneumococci due to low IgM antibody response to pneumococcal vaccinations. Infect Immun. 2011 Jan;79(1):314–20.
23. www.chest-ct-group.eu, assessed January 20, 2021.
24. Orange JS, Ballow M, Stiehm ER, Ballas ZK, Chinen J, De La Morena M, et al. Use and interpretation of diagnostic vaccination in primary immunodeficiency: a working group report of the Basic and Clinical Immunology Interest Section of the American Academy of Allergy, Asthma & Immunology. J Allergy Clin Immunol. 2012 Sep;130(3 Suppl):S1-24.
25. Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. J Chiropr Med. 2016 Jun;15(2):155–63.
26. Ademokun A, Wu Y-C, Martin V, Mitra R, Sack U, Baxendale H, et al. Vaccination-induced changes in human B-cell repertoire and pneumococcal IgM and IgA antibody at different ages. Aging Cell. 2011 Dec;10(6):922–30.
27. Simell B, Nurkka A, Ekström N, Givon-Lavi N, Käyhty H, Dagan R. Serum IgM antibodies contribute to high levels of opsonophagocytic activities in toddlers immunized with a single dose of the 9-valent pneumococcal conjugate vaccine. Clin Vaccine Immunol. 2012 Oct;19(10):1618–23.
28. Zhang Q, Arnaoutakis K, Murdoch C, Lakshman R, Race G, Burkinshaw R, et al. Mucosal immune responses to capsular pneumococcal polysaccharides in immunized preschool children and controls with similar nasal pneumococcal colonization rates. Pediatr Infect Dis J. 2004 Apr;23(4):307–13.
29. Orami T, Ford R, Kirkham L-A, Thornton R, Corscadden K, Richmond PC, et al. Pneumococcal conjugate vaccine primes mucosal immune responses to pneumococcal polysaccharide vaccine booster in Papua New Guinean children. Vaccine. 2020 Nov;38(50):7977–88.
30. Boyaka PN. Inducing Mucosal IgA: A Challenge for Vaccine Adjuvants and Delivery Systems. J Immunol. 2017 Jul;199(1):9–16.



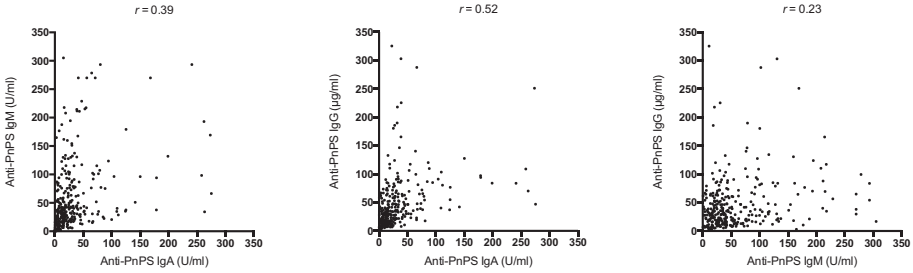
Supplementary Figure 9.1. Age-stratified titres for A) anti-PnPS IgM, B) anti-PnPS IgA, and C) anti-PnPS IgG.

Supplementary Figure 9.1. Specific antibody concentrations were defined in a patient population with suspected PID vaccinated with PnPS vaccine at pre-immunisation and 4-8 weeks and >8 weeks post-immunisation. Pre-immunisation samples: 0-20 years, n=39; 21-40 years, n=39; 41-60 years, n=51; 61-80 years, n=44; 4-8 weeks post-immunisation samples: 0-20 years, n=4; 21-40 years, n=5; 41-60 years, n=9; 61-80 years, n=8; >8 weeks post-immunisation samples: 0-20 years, n=12; 21-40 years, n=5; 41-60 years, n=7; 61-80 years, n=18. Box and Whisker plots show median concentrations, interquartile range and minimum/maximum values.

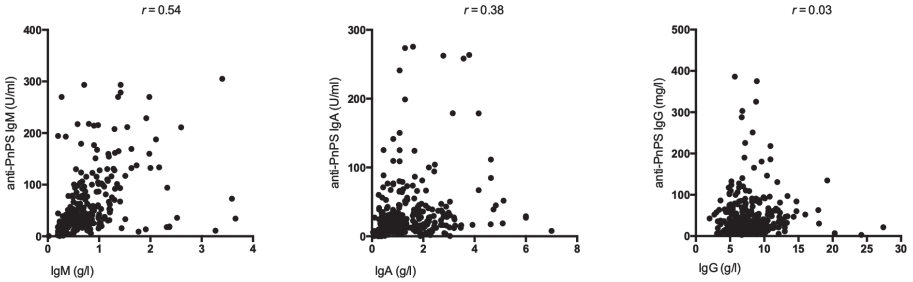


Supplementary Figure 9.2. Pre-immunisation age-stratified concentrations in Pn-C pre-vaccinated patients with suspected PID for anti-PnPS IgA, IgM and IgG.

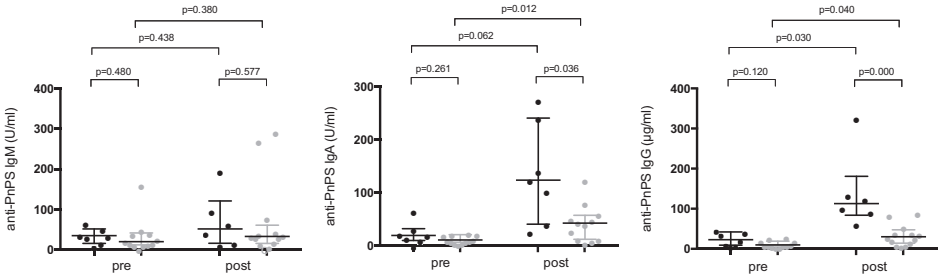
Supplementary Figure 9.2. Box and Whisker plots show median concentrations, interquartile range and minimum/maximum values.



Supplementary Figure 9.3. Spearman correlations (two-tailed) between the pneumococcal immunoglobulins.

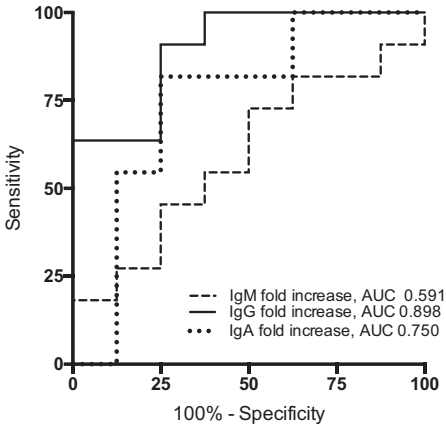


Supplementary Figure 9.4. Spearman correlations (two-tailed) between total immunoglobulin levels and pneumococcal immunoglobulins.



Supplementary Figure 9.5. Pre- and post-immunisation cumulative anti-PnPS IgM, IgA, and IgG titres for good (black dots) and poor (grey dots) responders as assessed by serotype-specific PnPS IgG testing in patients of whom both pre- and post-immunisation titres were available.

Supplementary Figure 9.5. P-values were calculated with Mann-Whitney U tests.



Supplementary Figure 9.6. Receiver operating characteristic (ROC) curves of sensitivity vs. specificity for the fold increase of anti-PnPS IgG, IgA and IgM vs. serotype-specific IgG response to vaccination.

Chapter 10

General discussion

GENERAL DISCUSSION

Recurrent ear-nose-throat (ENT) and lower airway symptoms commonly occur and have a negative impact on the quality of life for patients experiencing these symptoms. Such infections by themselves are generally innocent and self-limiting; only in a minority of patients are they a sign of underlying primary immunodeficiency (PID). Of all PIDs, hypogammaglobulinaemias form the largest group, but they are still quite rare, which makes diagnosis challenging. However, diagnostic delay may be critical for patients, as they may not receive appropriate treatment in a timely fashion, resulting in irreparable organ damage such as bronchiectasis. An important reason for diagnostic delay is the poor specificity of initial presenting symptoms, which are not recognised as indicators of potential underlying hypogammaglobulinaemia. Even when these patients are immunologically screened, investigations are often incomplete. In addition, some of the immunological laboratory investigations are difficult to interpret and non-immunologists often have little knowledge about these tests. To complicate things further, the definition of antibody deficiency depends on the local or regional reference range applicable to the patients and full international consensus regarding the definitions of the different forms of hypogammaglobulinaemia does not exist [1].

The aim of this thesis was to improve earlier detection of hypogammaglobulinaemia by investigating 1) clinical presentation patterns of single and multicentre cohorts of patients with hypogammaglobulinaemia and 2) easier-to-interpret diagnostic tests. Our meta-analysis reveals the importance of including immune dysregulation features to the currently existing infection-centred warning signs for hypogammaglobulinaemia. In addition to these medical aspects, non-medical aspects, such as coping strategies of patients who are 'always ill' and 'worn out', can help to distinguish hypogammaglobulinaemia from other diagnoses such as 'only innocent and self-limiting infections' (but not recognising the importance of their recurrence), or chronic fatigue syndrome (based on the 'worn out' feeling of afflicted patients). Our decision tree, which uses the more widely available and easy-to-interpret 23-valent pneumococcal IgG assay as a first-line test and the specialised serotype-specific assay as a second-line test, can be used as a reliable screening tool to identify patients with deficient polysaccharide antibody responses.

CLINICAL RECOGNITION OF HYPOGAMMAGLOBULINAEMIA

Primary care physicians, internists, and paediatricians are in the best position to initiate diagnostic evaluation of potential hypogammaglobulinaemia. Their awareness of this rare condition is therefore critical to reduce diagnostic delay [2]. The Jeffrey Modell Foundation (JMF) developed 'the 10 warning signs' of PID in an attempt to promote awareness and facilitate early recognition of PIDs; these have been revised twice, most recently in 2010 [3,4]. There are two versions, resulting from expert consensus: one for children, and one for adults. The warning signs focus on the type, number, severity and localisation of infections, their response to therapy and impact on growth, and on the family history. In spite of these warning signs, delayed diagnosis of PID remains a problem.

The JMF warning signs of PID are mainly infection-focussed, while other symptoms may be more prominent at first, such as autoimmune manifestations, and lymphoid or granulomatous diseases [5,6]. Data from the national French PID registry e.g. showed that 26% of PID patients had experienced at least one manifestation of immune dysregulation during their lifetime, of which autoimmune cytopenias and inflammatory bowel disease occurred most frequently [7]. Mauracher et al. showed that immune dysregulation can also be the only manifestation of PID without any signs of infection [8], and Thalhammer et al. showed that an exclusive focus on infection-centred warning signs would have missed around 25% of PID patients [9]. The multi-stage diagnostic protocol, developed by the Clinical Working Party of the European Society for Immunodeficiencies (ESID) includes the non-infectious manifestations; it takes the clinical presentation of the patient as starting point [10]. Eight different clinical presentations determined by the underlying pathology of the disease were identified by expert consensus. Next to infection-focussed presentation patterns (recurrent ENT and airway infections, recurrent pyogenic infections, unusual infections or unusually severe course of infections, recurrent infections with the same type of pathogen), this protocol includes autoimmune or chronic inflammatory disease as a clinical presentation pattern [10,11]. Adding immune dysregulation manifestations to the JMF warning signs has been suggested by several studies, but these suggestions were not specifically investigated in hypogammaglobulinaemia patients [2,12–14]. In *chapter 5,* we assessed initial clinical manifestations in CVID patients in a meta-analysis. We demonstrated that also in CVID patients other presenting manifestations than frequent and/or severe infections often occur, such as lymphadenopathy (27%), splenomegaly (13%), chronic diarrhoea (21%), inflammatory bowel disease (11%), autoimmune cytopenia (10%) and idiopathic thrombocytopenia (6%) [15]. Not only the infectious but also the immune dysregulation features should therefore alert to the possibility of CVID, regardless of whether these features occur with or without recurrent infections. However, with the awareness of the full spectrum of all possible medical presentation patterns, we are not there yet. For example, recurrent upper/lower respiratory tract infections are much more prevalent without concomitant hypogammaglobulinaemia.

In *chapter 7* we show that strategies of hypogammaglobulinaemic patients developed to cope with the recurrent infections and extreme fatigue differ from those of patients with chronic fatigue syndrome [16]. While patients with chronic fatigue syndrome often use escape / avoidance strategies, patients with hypogammaglobulinaemia tend to normalise their symptoms and carry on with usual activities [16,17]. This example shows that to timely identify hypogammaglobulinaemia 'pattern recognition' should not only focus on the medical 'red flags', but also on information outside the medical domain such as these coping strategies of PAD patients who present being 'always ill' and 'worn out'.

CLINICAL RELEVANCE OF NON-CVID HYPOGAMMAGLOBULINAEMIA

The term CVID was introduced in 1971 to distinguish patients with less well-defined hypogammaglobulinaemia from those with a consistent clinical phenotype [18]. However, the definition of CVID remains a topic of ongoing debate. Disagreement exists about whether or not to include a mandatory decrease in IgA in the definition of CVID [1] and about the approval of reduced (switched) memory B lymphocytes as an alternative criterion to conclude vaccine responses are impaired [19,20]. A range of other combinations of antibody deficiencies where the CVID definitions are not met can be encountered (decreased total IgG, IgG-subclass(es), IgM, IgA and/or specific antibodies alone or in combination). Various terminologies have been used for such patients in the literature, also depending on their degree of perceived disease severity: idiopathic primary hypogammaglobulinaemia [21], CVID-like disorder [22], IgG isotype deficiency [23], and unclassified hypogammaglobulinaemia [24]. We refer to this category as 'unclassified primary antibody deficiency' (unPAD). While the clinical spectrum of CVID has been investigated in depth (reviewed in *chapter 5*), there are relatively few reports on patients with these milder forms of hypogammaglobulinaemia who show clinical features reminiscent of CVID to a greater or lesser extent. This negatively affects this group of hypogammaglobulinaemic patients who are in fact much more often encountered in clinical practice. On the one hand, patients with subnormal immunoglobulin levels and in particular subnormal IgG subclass levels are sometimes asymptomatic [25,26]. On the other hand, unPAD patients can evolve into a complete CVID phenotype over time [1]. The clinically most important difference between CVID and unPAD is that patients with CVID are more prone to develop severe autoimmunity, interstitial lung disease, granulomatous infiltrations, lymphoid hyperplasia, and lymphoid malignancies, as compared to unPAD patients [23]. In *chapter 2* we show in a relatively large (symptomatic) unPAD cohort that these patients present heterogenous manifestations encompassing a wide range of disease severity. While some patients suffered from unusual infections / unusually severe course of infections or even autoimmune or chronic inflammatory disease, others only suffered from recurrent 'normal' ENT and airway infections or chronic fatigue [27]. A striking 44% already showed bronchiectasis at presentation, and their health-related quality of life (HRQoL) was significantly decreased in all domains, meaning that a lot of unPAD patients had to cope simultaneously with pain, negative feelings and impairments in cognition, home management tasks, sleep, social interaction, and work. Although unPAD is generally considered clinically mild and not very relevant, these results show that patients with unPAD can suffer from serious conditions and impairment, and that it is important to pay more attention to the potential disease burden of unPADs.

For example, when a repeatedly isolated decreased serum IgM is found, i.e. selective IgM deficiency (sIgMdef), clinicians are also confronted with a dilemma. The clinical consequences of sIgMdef are not sufficiently known, and therefore clinicians struggle with

what they should do with such a finding. IgM deficiency has been linked to a variety of clinical manifestations, including severe or recurrent infections, atopy, autoimmunity and malignancy [28–34]. However, these studies have been mainly performed in tertiary centres and are therefore biased towards disease. In *chapter 3* we showed that decreased serum IgM levels can often incidentally be found in asymptomatic adults in a secondary centre population [35]. Determination of the clinical significance of sIgMdef is not only challenged by the rarity and highly variable phenotype, but – analogous to the dispute about the correct definition of CVID – also by the different criteria for 'selective IgM deficiency' that are used in the literature [29–31,36–38]. In 2017, the IUIS defined sIgMdef as an absent serum IgM level [39], and the ESID online Registry as serum IgM levels repeatedly below 2 standard deviations of normal with normal levels of serum IgA and IgG and IgG subclasses, normal vaccine responses, absence of T lymphocyte defects, and absence of causative external factors (www.esid.org). Many previously published papers that report on 'IgM deficiency' also include IgM-deficient patients with decreased IgG-subclass(es), abnormal vaccine response and/or T lymphocyte defects under the term 'selective IgM deficiency'. We showed in *chapter 3* that in a quarter of the literature cases, the deficiency is not 'selective', because other immunological abnormalities were present. These patients with concomitant specific antibody deficiency (SPAD) and/or IgG subclass deficiencies may be at risk of more severe and frequent infections, comparable to the increased number of lower respiratory tract infections and bronchiectasis in patients with IgA deficiency in combination with IgG subclass deficiency and/or SPAD [15]. Patients with recurrent and/or severe infections and decreased serum IgM levels in combination with SPAD have been described to benefit from immunoglobulin substitution [4,16]. Therefore, we prefer to categorise these IgM-deficient patients with concomitant other immunological abnormalities as unPAD. In order to answer the question: "How should we manage patients with repeatedly isolated decreased serum IgM levels?", we performed a large multicentre study with the aim to only include *truly* sIgMdef patients (*chapter 4*). Unfortunately, even this multi-centre study could not answer the question. When a persistently decreased serum IgM was found, it was rarely fully analysed (data on IgG subclasses and/or vaccination responses were lacking). If we want to truly answer the question how to manage patients with repeatedly isolated decreased serum IgM levels we should fully analyse and accurately describe these patients.

In addition to our studies on isolated decreased serum IgM, we also studied the clinical relevance of isolated impaired anti-pneumococcal polysaccharide (PnPS) IgG, IgM and IgA responses. The published clinical manifestations of SPAD are mostly based on IgG polysaccharide antibody deficiency and comprise recurrent respiratory tract infections with encapsulated bacteria, and less often autoimmune or rheumatic diseases, chronic diarrhoea, and bronchiectasis [40]. The clinical relevance of specific PnPS IgM or IgA deficiency is as yet unknown. Theoretically, both specific PnPS IgM and specific PnPS IgA deficiency could be clinically relevant, because both IgM and IgA antibodies are part of mucosal immunity

and can prevent bacterial adhesion and thus colonisation of the upper respiratory tract epithelium [41]. These antibodies could therefore act as epithelial surfaces to clear bacteria and prevent colonisation and invasion. IgA is the predominant immunoglobulin isotype in the mucosal immune system, which widely exists in the gastrointestinal tract, respiratory tract, vaginal tract, tears, saliva, and colostrum [42]. Thus, IgA is critical for mucosal immunity and maintenance of the intestinal microbial homeostasis. Of the antibody-producing cells in mucosae, IgM-producing cells account for 6% (nasal glands) to 18% (duodenum/jejunum) [41]. It is therefore plausible that IgM antibodies may, at least in part, protect patients against bacteria known to colonise the upper and lower respiratory tract epithelium, such as *Streptococcus pneumoniae* [43]. In patients with hyper IgM syndrome a protective role of IgM anti-*H influenzae* antibodies in both sera and saliva has been suggested to reduce the risk of acute infections and chronic respiratory tract disease in these patients [44]. Indeed, in patients with established CVID [45,46] and PAD [47] decreased anti-PnPS IgA and IgM responses have been associated with a greater rate of respiratory infections. In *chapter 9* we show that all patients in our cohort with a decreased anti-PnPS IgM or IgA response had already been classified as having a form of PAD based on serotype-specific PnPS IgG testing and serum immunoglobulin levels [48]. Therefore, we could not demonstrate the clinical relevance of 'specific anti-pneumococcal IgM or IgA antibody deficiency' based on our data. It is useful to further investigate this issue in a larger cohort.

We do not know why an affected individual develops a particular unPAD phenotype. Furthermore, the extensive disease heterogeneity complicates research in this area. While several classifications have been proposed to distinguish subgroups among CVID patients, mainly based on peripheral B and/or T lymphocytes [49–53] and clinical phenotypes [54], this has not yet been investigated for unPAD patients. We made, for the first time, an attempt to subclassify unPAD in *chapter 2*. Unfortunately, due to the limited sample size (99 adult patients), this was not (yet) feasible. In our still ongoing 'unPAD study' (described in detail in *chapter 6*) we will focus on subclassifying unPAD, not only to predict disease outcomes, but also to inform which subgroups should be more strictly monitored or differently treated according to subtype. In addition, the potential identification of more homogeneous subgroups can help to unravel the genetic background of unPAD patients. This information will help to guide clinicians to answer the question: "what should I do with this individual unPAD patient?".

LABORATORY EVALUATION OF HYPOGAMMAGLOBULINAEMIC PATIENTS

Two important functions of antibodies in the immune response to infection are the neutralisation of viruses and opsonisation of bacteria. Assays that are fully able to assess these functions are not available for routine clinical use. Instead, antibody function is estimated by measuring an individual's response to specific vaccinations. The measurement of vaccine responses is indicated when upper/lower respiratory tract infections occur frequently, infections are unusual or severe, or when there is an unusual need for antibiotic treatment. According to the ESID protocol for diagnosing PID, serum IgA, IgG and IgM should first be determined to rule out severe antibody deficiency [11]. In case of a decreased level of at least one isotype or if the results are normal but recurrent ENT and airway infections persist for more than 3-6 months, vaccine responses are assessed. Where the protein vaccines – e.g. tetanus and diphtheria vaccines – require intact B and T lymphocyte function, polysaccharide vaccines – e.g., 23-valent pneumococcal polysaccharide (PnPS) vaccine – require functional B lymphocytes only. SPAD is characterized by deficient antibody production against the capsular polysaccharides of encapsulated bacteria, such as *Streptococcus pneumoniae*. The diagnosis of SPAD is classically based on the measurement of serotype-specific IgG against PnPS vaccines [55]. The lack of consensus and of evidence regarding the diagnostic criteria of SPAD is a challenge in daily clinical practise [56]. Serotype-specific concentrations ≥1.3 mcg/mL are indicative of normal ability to respond to polysaccharide antigens, but lower levels (≥0.35 mcg/ml) are considered adequate for protection against invasive pneumococcal disease [55,57]. Discussion exists about the amount of serotypes that have to achieve these values; some experts believe that the normal response should be reduced to > 50% of the tested serotypes rather than >70% of the tested serotypes [58,59]. An additional problem is that many health care providers do not have access to perform the serotype-specific PnPS assay [60–62]. Other tests have been proposed as complementary to or as alternative for the PnPS response for the diagnosis of SPAD, such as measurement of anti-*Salmonella (S.) typhi* Vi antibodies, and of the cumulative IgG response to all 23 serotypes present in the PnPS vaccine (23-valent IgG assay). Indeed, in *chapter* 8, we showed that the 23-valent IgG assay is a reliable screening test for identifying conjugated-vaccine-naive patients who respond normally to PnPS vaccination in a low pre-test probability setting. This assay is therefore particularly useful in general hospitals. By filtering out those patients with recurrent infections who probably do not have antibody deficiency - which is by far the largest group in such a setting - fewer patients will have to be referred to specialised centres for serotype testing. This highlights the importance of performing diagnostic tests suited to the clinical setting. The IgG response to Typhim Vi vaccination, using the VaccZyme *Salmonella typhi* Vi IgG ELISA, has also been shown to be of additional use to accompany serotype-specific PnPS responses for the assessment of antibody deficiencies [63–66]. In contrast to PnPS antibodies,

the concentrations of Typhim Vi antibodies are low in most healthy Western citizens [65]. The measurement of an IgG response to the Typhim Vi vaccine is therefore especially useful for patients suspected of having an antibody deficiency who have a high baseline concentration of pneumococcal antibodies or in patients who have previously received a conjugated pneumococcal vaccine or immunoglobulin substitution therapy. In *chapter 9*, we investigated whether addition of the anti-PnPS IgA and/or IgM assay could reduce the need for serotype-specific IgG testing [48]. However, adding these assays to the anti-PnPS IgG assay could not reduce the need for the more expensive and difficult to interpret serotype-specific IgG testing in our general hospital cohort.

THE GENETICS OF HYPOGAMMAGLOBULINAEMIA: ONGOING DEVELOPMENT

The aetiology of hypogammaglobulinaemia is largely unknown. Many cases seem to be complex disorders in which multiple genes and/or environmental factors determine the final phenotype [1]. Increasingly, but still only in a minority of patients with CVID (< 20% in nonconsanguineous cohorts [1]; ± 70% in consanguineous cohorts [67]) specific genetic defects have been identified (e.g. mutations in genes involved in signalling through the B-cell receptor (CD19, CD21, CD81), and those involved in costimulatory pathways necessary for isotype switching and somatic hypermutation during B-lymphocyte activation (ICOS, BAFFR, NF-kB1, TACI)) [68–71]. Alterations in the TNFRSF13B (TACI) gene are, however, not only found in CVID patients; they have also been reported in patients with IgG subclass deficiencies [72,73] and IgA deficiency [74]. Alterations in TACI are no longer regarded as disease causing but as cofactors affecting especially the T lymphocyte-independent antibody response and increasing the risk of developing autoimmune and lymphoproliferative complications. Other localised genetic defects in CVID patients have been identified in genes such as lipopolysaccharide responsive beige-like anchor protein (LRBA) [75], CTLA4 [76] and PIK3CD [77]. Nowadays, each defective molecule is considered a separate form of immunodeficiency and is considered a monogenic disorder. Schouwenburg et al. identified variants in CVID genes TNFRSF13C, LRBA and NLRP12 and enrichment of variants in known and novel disease pathways, confirming a polygenic nature of CVID and individual-specific aetiologies [78]. In addition, recent studies demonstrated the role of epigenetic modifications in the development of disorders associated with CVID [79,80]. These epigenetic mechanisms can influence gene expression without altering the germline DNA gene sequences and play an important role in the normal developmental program of immune cells [80]. The mechanisms described to date include DNA methylation, chromatin modulation, histone modification, transcription factor expression, and noncoding RNAs [81]. Multiple reports support the notion of a complex basis of CVID and related milder PAD disorders, in which an accumulation of multiple genetic and/or environmental factors contributes to the final phenotype [79,82,83]. Long-term follow-up of currently healthy family members who are carrying known CVID-related genetic variants can help identify epigenetic or environmental factors that influence the clinical penetrance of these variants.

Our studies suggest that X-linked mechanisms may play a role in the development of some forms of primary hypogammaglobulinaemia. In both our cohort of unPAD patients (*chapter 2*), our review of CVID patients (*chapter 5*) and previously published ESID online Registry reports [84–86], males predominated in children (74% in unPAD; 62% in CVID). This could be the result of (unrecognised) X-linked disease in these patients. On the contrary, females predominated in adults (74% in unPAD; 58% in CVID), suggesting that

these diseases are not the same in children and adults. Adult female predominance also suggests that sex hormone effects, environmental exposure and epigenetic influences might play a role [87]. These findings in any case implicate that future studies attempting to define disease mechanisms should be stratified according to sex and age of disease onset.

FUTURE PERSPECTIVES

This thesis describes several aspects of patients with PAD, with a special focus on unPAD: the clinical picture, both at presentation and during follow-up, the current diagnostic work-up practices, new screening methods enabling earlier detection, and the impact of unPAD on patients' daily lives. To further improve early detection, future studies may include the development of pattern recognition algorithms, because it is impossible for non-experts to know about all >8000 rare diseases; physicians are not likely to recognise a pattern they have (almost) never encountered. Thus, to minimise diagnostic delay, doctors need help. Since the 1960s attempts have been made to develop computer-aided diagnosis support systems (DSSs) [88–91]. Examples are DXplain [92], GIDEON (Global Infectious Diseases and Epidemiology Network) [93], and Isabel [94], but their routine clinical use remains limited. In DXplain a set of clinical findings (signs, symptoms, laboratory data) can be entered to produce a ranked list of diagnoses which might explain the clinical manifestations [92]. GIDEON was developed for the fields of geographic and travel medicine and can generate a ranked differential diagnosis based on signs, symptoms, laboratory results, country of origin and incubation period [93]. Isabel, named after the gravely ill daughter of the founder, consists of a diagnosis checklist system, and includes more than 11.000 diagnoses [94]. In a recent systematic review, differential diagnostic generators were reported to achieve high levels of accurate diagnosis, but there was no evidence that they performed better than clinicians [95]. They are often considered impractical in clinical practice due to the large number of suggested possible diagnoses. Weber et al. noted that big data can really support transformations in health care when data sets can be linked at the individual person level [96]. Technology may now be ripe to enable the development of next-generation DSSs based on these key insights.

Future studies should also be directed towards better characterisation and classification of the disease and understanding of the mechanisms that cause primary antibody deficiency. UnPAD is a highly heterogeneous disease group and will remain so unless we succeed to classify the group into clinically meaningful subgroups. Efforts to stratify patients into different subgroups according to genetic screening, B and T lymphocyte studies and clinical presentations have already been made for CVID patients. These have resulted in new ways to diagnose and treat CVID. However, these efforts have not yet been made for unPAD patients. Next to characterising unPAD, the unPAD study (*chapter 6*) has been designed to investigate this for unPAD patients. A large, multicentre ESID online Registry cohort will be collected; all data will be fully monitored to ensure data quality. Data will comprise the demographical, clinical and immunological characteristics of these patients at diagnosis and during follow-up. Due to the large size of the cohort, it will become feasible to perform meaningful subgroup analyses based on the collected characteristics. These – by nature more homogeneous – subgroups may help to unravel the genetic background of unPAD patients in the future by opening new perspectives in the analysis of gene mutations and alterations that may help to

identify patients at high risk of unPAD complications at diagnosis and provide information about underlying disease mechanisms, thereby guiding the best choice of therapy. A good example for patients primarily diagnosed with CVID is the discovery of gain-of-function or loss-of-function mutations in the NF-kB pathway [97]. This pathway plays an important role in multiple biological processes, such as development and architectural organization of secondary lymphoid organs, B lymphocyte maturation and survival, B-lymphocyte-mediated immune responses and antibody production, formation of germinal centres, and various roles in T lymphocyte responses [98]. Not only can unPAD classification facilitate research on disease causing mechanisms and the targeted search and identification of genetic defects, it also has potential to guide follow-up schedules and tailor treatment strategies for identified subgroups of unPAD patients. This will ultimately shed light on more personalised intervention approaches. However, as already seen in patients with CVID, it is plausible that phenotypic heterogeneity within patients with the same genetic variant(s) will complicate the identification of genetic factors, because of variable severity of the defect or other unknown factors.

FINAL CONCLUSIONS OF THE THESIS

1. Mild hypogammaglobulinaemia can cause serious morbidity and often leads to significant impairment of health-related quality of life
2. Truly selective IgM deficiency is probably very rare

3A. As previously recognised, most patients with CVID present with recurrent respiratory infections, but our meta-analysis demonstrates that CVID patients also frequently present with severe bacterial infections (meningitis, septicaemia, mastoiditis, osteomyelitis) and immune dysregulation features (chronic lymphadenopathy, splenomegaly, inflammatory bowel disease, autoimmune cytopenia and idiopathic thrombocytopenia), identifying patient categories that may also require evaluation for CVID

3B. Presentation of CVID is characterised by a bimodal sex distribution with male predominance in children (62%) and female predominance in adults (58%) suggesting differences in paediatric- and adult-onset CVID aetiology

4A. Patients diagnosed with PAD demonstrate a specific pattern of complaint presentation; they tend to normalize their symptoms (e.g. fatigue and recurrent infections) and to carry on with usual activities, which is different from patients with for example chronic fatigue syndrome, who tend to use avoidance strategies

4B. Multiple factors negatively affect the timely diagnosis of PAD: misattribution of the presenting symptoms, getting so used to symptoms that they are considered to be 'normal', the feeling of not being taken seriously, and no longer seeking care because there you are 'just being treated for symptoms' instead of being investigated for their cause

5A. In a low pre-test probability setting, the 23-valent Pneumococcal IgG assay is a reliable screening test to identify probable good responders in vaccine naïve patients

5B. Patients classified as having an intact IgG PnPS antibody response can still display decreased anti-PnPS IgA and IgM responses

LITERATURE

1. Bonilla FA, Barlan I, Chapel H, Costa-Carvalho BT, Cunningham-Rundles C, de la Morena MT, et al. International Consensus Document (ICON): Common Variable Immunodeficiency Disorders. J allergy Clin Immunol Pract. 2016;4(1):38–59.
2. Arkwright PD, Gennery AR. Ten warning signs of primary immunodeficiency: a new paradigm is needed for the 21st century. Ann N Y Acad Sci. 2011 Nov;1238:7–14.
3. http://www.info4pi.org.
4. Modell V, Gee B, Lewis DB, Orange JS, Roifman CM, Routes JM, et al. Global study of primary immunodeficiency diseases (PI)--diagnosis, treatment, and economic impact: an updated report from the Jeffrey Modell Foundation. Immunol Res. 2011 Oct;51(1):61–70.
5. Grimbacher B, Warnatz K, Yong PFK, Korganow A-S, Peter H-H. The crossroads of autoimmunity and immunodeficiency: Lessons from polygenic traits and monogenic defects. J Allergy Clin Immunol. 2016 Jan;137(1):3–17.
6. Cunningham-Rundles C. Autoimmunity in primary immune deficiency: taking lessons from our patients. Clin Exp Immunol. 2011 Jun;164 Suppl(Suppl 2):6–11.
7. Fischer A, Provot J, Jais J-P, Alcais A, Mahlaoui N. Autoimmune and inflammatory manifestations occur frequently in patients with primary immunodeficiencies. J Allergy Clin Immunol. 2017 Nov;140(5):1388-1393.e8.
8. Mauracher AA, Gujer E, Bachmann LM, Güsewell S, Pachlopnik Schmid J. Patterns of Immune Dysregulation in Primary Immunodeficiencies: A Systematic Review. J allergy Clin Immunol Pract. 2021 Feb;9(2):792-802.e10.
9. Thalhammer J, Kindle G, Nieters A, Rusch S, Seppänen MRJ, Fischer A, et al. Initial presenting manifestations in 16,486 patients with inborn errors of immunity include infections and noninfectious manifestations. J Allergy Clin Immunol. 2021 Apr;
10. De Vries E. Patient-centred screening for primary immunodeficiency: A multi-stage diagnostic protocol designed for non-immunologists. Clin Exp Immunol. 2006;
11. de Vries E. Patient-centred screening for primary immunodeficiency, a multi-stage diagnostic protocol designed for non-immunologists: 2011 update. Clin Exp Immunol. 2012 Jan;167(1):108–19.
12. Bjelac JA, Yonkof JR, Fernandez J. Differing Performance of the Warning Signs for Immunodeficiency in the Diagnosis of Pediatric Versus Adult Patients in a Two-Center Tertiary Referral Population. J Clin Immunol. 2019 Jan;39(1):90–8.
13. Arslan S, Ucar R, Caliskaner AZ, Reisli I, Guner SN, Sayar EH, et al. How effective are the 6 European Society of Immunodeficiency warning signs for primary immunodeficiency disease? Ann Allergy Asthma Immunol. 2016 Feb;116(2):151-155.e1.
14. Lankisch P, Schiffner J, Ghosh S, Babor F, Borkhardt A, Laws H-J. The Duesseldorf warning signs for primary immunodeficiency: is it time to change the rules? J Clin Immunol. 2015 Apr;35(3):273–9.
15. Janssen LMA, van der Flier M, de Vries E. Lessons Learned From the Clinical Presentation of Common Variable Immunodeficiency Disorders: A Systematic Review and Meta-Analysis. Front Immunol. 2021;12:620709.
16. Janssen LMA, van den Akker K, Boussihmad MA, de Vries E. Which triggers could support timely identification of primary antibody deficiency? A qualitative study using the patient perspective. Orphanet J Rare Dis. 2021 Jun;16(1):289.
17. Johnson SK, DeLuca J, Natelson BH. Assessing somatization disorder in the chronic fatigue Syndrome. Psychosom Med. 1996;

18. Fudenberg H, Good RA, Goodman HC, Hitzig W, Kunkel HG, Roitt IM, et al. Primary immunodeficiencies. Report of a World Health Organization Committee. Pediatrics. 1971 May;47(5):927–46.
19. Ameratunga R, Woon S-T, Gillis D, Koopmans W, Steele R. New diagnostic criteria for common variable immune deficiency (CVID), which may assist with decisions to treat with intravenous or subcutaneous immunoglobulin. Clin Exp Immunol. 2013 Nov;174(2):203–11.
20. Ameratunga R, Brewerton M, Slade C, Jordan A, Gillis D, Steele R, et al. Comparison of diagnostic criteria for common variable immunodeficiency disorder. Front Immunol. 2014;5:415.
21. Driessen GJ, Dalm VASH, van Hagen PM, Grashoff HA, Hartwig NG, van Rossum AMC, et al. Common variable immunodeficiency and idiopathic primary hypogammaglobulinemia: two different conditions within the same disease spectrum. Haematologica. 2013 Oct;98(10):1617–23.
22. van de Ven AAJM, van Montfrans JM. Clinical complications in pediatric CVID are not restricted to patients with severely reduced class-switched memory B cells. Vol. 22, Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. England; 2011. p. 347–8.
23. Filion CA, Taylor-Black S, Maglione PJ, Radigan L, Cunningham-Rundles C. Differentiation of Common Variable Immunodeficiency From IgG Deficiency. J allergy Clin Immunol Pract. 2019 Apr;7(4):1277–84.
24. Kutukculer N, Gulez N. The outcome of patients with unclassified hypogammaglobulinemia in early childhood. Pediatr Allergy Immunol. 2009 Nov;20(7):693–8.
25. Durandy A, Kracker S, Fischer A. Primary antibody deficiencies. Nat Rev Immunol. 2013 Jul;13(7):519–33.
26. Fried AJ, Bonilla FA. Pathogenesis, Diagnosis, and Management of Primary Antibody Deficiencies and Infections. Clin Microbiol Rev. 2009;22(3):396–414.
27. Janssen LMA, Bassett P, Macken T, van Esch J, Pruijt H, Knoops A, et al. Mild Hypogammaglobulinemia Can Be a Serious Condition. Front Immunol. 2018;9:2384.
28. Louis AG, Gupta S. Primary selective IgM deficiency: an ignored immunodeficiency. Clin Rev Allergy Immunol. 2014 Apr;46(2):104–11.
29. Goldstein MF, Goldstein AL, Dunsky EH, Dvorin DJ, Belecanech GA, Shamir K. Pediatric selective IgM immunodeficiency. Clin Dev Immunol. 2008;2008:624850.
30. Goldstein MF, Goldstein AL, Dunsky EH, Dvorin DJ, Belecanech GA, Shamir K. Selective IgM immunodeficiency: retrospective analysis of 36 adult patients with review of the literature. Ann Allergy Asthma Immunol. 2006 Dec;97(6):717–30.
31. Chovancova Z, Kralickova P, Pejchalova A, Bloomfield M, Nechvatalova J, Vlkova M, et al. Selective IgM Deficiency: Clinical and Laboratory Features of 17 Patients and a Review of the Literature. J Clin Immunol. 2017 Aug;37(6):559–74.
32. Lucuab-Fegurgur DL, Gupta S. Comprehensive clinical and immunological features of 62 adult patients with selective primary IgM deficiency. Am J Clin Exp Immunol. 2019;8(6):55–67.
33. Caka C, Cimen O, Kahyaoğlu P, Tezcan İ, Cagdas D. Selective IgM deficiency: Follow-up and outcome. Pediatr allergy Immunol Off Publ Eur Soc Pediatr Allergy Immunol. 2021 Aug;32(6):1327–34.
34. Ni J, Zhang J, Chen Q, Chen Y, Liu J. The epidemiology and clinical features of selective immunoglobulin M deficiency: A single-center study in China. J Clin Lab Anal. 2020 Jul;34(7):e23289.
35. Janssen LMA, Macken T, Creemers MCW, Pruijt JFM, Eijk JJJ, De Vries E. Truly selective primary IgM deficiency is probably very rare.
36. Yel L, Ramanuja S, Gupta S. Clinical and immunological features in IgM deficiency. Int Arch Allergy Immunol. 2009;150(3):291–8.

37. Louis AG, Agrawal S, Gupta S. Analysis of subsets of B cells, Breg, CD4Treg and CD8Treg cells in adult patients with primary selective IgM deficiency. Am J Clin Exp Immunol. 2016;5(1):21–32.
38. Gupta S, Gupta A. Defining Primary Selective IgM Deficiency. Vol. 39, Journal of clinical immunology. Netherlands; 2019. p. 350–2.
39. Bousfiha A, Jeddane L, Picard C, Ailal F, Bobby Gaspar H, Al-Herz W, et al. The 2017 IUIS Phenotypic Classification for Primary Immunodeficiencies. J Clin Immunol. 2018 Jan;38(1):129–43.
40. Cheng YK, Decker PA, O'Byrne MM, Weiler CR. Clinical and laboratory characteristics of 75 patients with specific polysaccharide antibody deficiency syndrome. Ann allergy, asthma Immunol Off Publ Am Coll Allergy, Asthma, Immunol. 2006 Sep;97(3):306–11.
41. Brandtzaeg P, Johansen F-E. Mucosal B cells: phenotypic characteristics, transcriptional regulation, and homing properties. Immunol Rev. 2005 Aug;206:32–63.
42. Chen K, Magri G, Grasset EK, Cerutti A. Rethinking mucosal antibody responses: IgM, IgG and IgD join IgA. Nat Rev Immunol. 2020 Jul;20(7):427–41.
43. Tsang RSW, Sill ML, Skinner SJ, Law DKS, Zhou J, Wylie J. Characterization of invasive Haemophilus influenzae disease in Manitoba, Canada, 2000-2006: invasive disease due to non-type b strains. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2007 Jun;44(12):1611–4.
44. Micol R, Kayal S, Mahlaoui N, Beauté J, Brosselin P, Dudoit Y, et al. Protective effect of IgM against colonization of the respiratory tract by nontypeable Haemophilus influenzae in patients with hypogammaglobulinemia. J Allergy Clin Immunol. 2012 Mar;129(3):770–7.
45. Cavaliere FM, Milito C, Martini H, Schlesier M, Dräger R, Schütz K, et al. Quantification of IgM and IgA anti-pneumococcal capsular polysaccharides by a new ELISA assay: a valuable diagnostic and prognostic tool for common variable immunodeficiency. J Clin Immunol. 2013 May;33(4):838–46.
46. Pulvirenti F, Milito C, Cavaliere FM, Mezzaroma I, Cinetto F, Quinti I. IGA Antibody Induced by Immunization With Pneumococcal Polysaccharides Is a Prognostic Tool in Common Variable Immune Deficiencies. Front Immunol. 2020;11:1283.
47. Echeverría de Carlos A, Gómez de la Torre R, García Carus E, Caminal Montero L, Bernardino Díaz López J, Suárez Casado H, et al. Concentrations of Pneumococcal IgA and IgM are compromised in some individuals with antibody deficiencies. J Immunoassay Immunochem. 2017;38(5):505–13.
48. Janssen LMA, Heron M, Murk J-L, Leenders ACAP, Rijkers GT, de Vries E. The clinical relevance of IgM and IgA anti-pneumococcal polysaccharide ELISA assays in patients with suspected antibody deficiency. Clin Exp Immunol. 2021 Aug;205(2):213–21.
49. Warnatz K, Denz A, Drager R, Braun M, Groth C, Wolff-Vorbeck G, et al. Severe deficiency of switched memory B cells (CD27(+)IgM(-)IgD(-)) in subgroups of patients with common variable immunodeficiency: a new approach to classify a heterogeneous disease. Blood. 2002 Mar;99(5):1544–51.
50. Piqueras B, Lavenu-Bombled C, Galicier L, Bergeron-van der Cruyssen F, Mouthon L, Chevret S, et al. Common variable immunodeficiency patient classification based on impaired B cell memory differentiation correlates with clinical aspects. J Clin Immunol. 2003 Sep;23(5):385–400.
51. Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, et al. The EUROclass trial: defining subgroups in common variable immunodeficiency. Blood. 2008 Jan;111(1):77–85.
52. Picat M-Q, Thiébaut R, Lifermann F, Delbrel X, Adoue D, Wittkop L, et al. T-cell activation discriminates subclasses of symptomatic primary humoral immunodeficiency diseases in adults. BMC Immunol. 2014 Mar;15:13.
53. Rösel AL, Scheibenbogen C, Schliesser U, Sollwedel A, Hoffmeister B, Hanitsch L, et al. Classification of common variable immunodeficiencies using flow cytometry and a memory B-cell functionality assay. J Allergy Clin Immunol. 2015 Jan;135(1):198–208.

54. Chapel H, Lucas M, Lee M, Bjorkander J, Webster D, Grimbacher B, et al. Common variable immunodeficiency disorders: division into distinct clinical phenotypes. Blood. 2008 Jul;112(2):277–86.
55. Orange JS, Ballow M, Stiehm ER, Ballas ZK, Chinen J, De La Morena M, et al. Use and interpretation of diagnostic vaccination in primary immunodeficiency: a working group report of the Basic and Clinical Immunology Interest Section of the American Academy of Allergy, Asthma & Immunology. J Allergy Clin Immunol. 2012 Sep;130(3 Suppl):S1-24.
56. Perez E, Bonilla FA, Orange JS, Ballow M. Specific Antibody Deficiency: Controversies in Diagnosis and Management. Front Immunol. 2017;8:586.
57. Siber GR, Chang I, Baker S, Fernsten P, O'Brien KL, Santosham M, et al. Estimating the protective concentration of anti-pneumococcal capsular polysaccharide antibodies. Vaccine. 2007 May;25(19):3816–26.
58. Kashani S, Carr TF, Grammer LC, Schleimer RP, Hulse KE, Kato A, et al. Clinical characteristics of adults with chronic rhinosinusitis and specific antibody deficiency. J allergy Clin Immunol Pract. 2015;3(2):236–42.
59. Chiarella SE, Grammer LC. Immune deficiency in chronic rhinosinusitis: screening and treatment. Expert Rev Clin Immunol. 2017 Feb;13(2):117–23.
60. Daly TM, Hill HR. Use and clinical interpretation of pneumococcal antibody measurements in the evaluation of humoral immune function. Clin Vaccine Immunol. 2015 Feb;22(2):148–52.
61. Balloch A, Licciardi P V, Tang MLK. Serotype-specific anti-pneumococcal IgG and immune competence: critical differences in interpretation criteria when different methods are used. J Clin Immunol. 2013 Feb;33(2):335–41.
62. Balmer P, Cant AJ, Borrow R. Anti-pneumococcal antibody titre measurement: what useful information does it yield? J Clin Pathol. 2007 Apr;60(4):345–50.
63. Sanchez-Ramon S, de Gracia J, Garcia-Alonso AM, Rodriguez Molina JJ, Melero J, de Andres A, et al. Multicenter study for the evaluation of the antibody response against salmonella typhi Vi vaccination (EMPATHY) for the diagnosis of Anti-polysaccharide antibody production deficiency in patients with primary immunodeficiency. Clin Immunol. 2016 Aug;169:80–4.
64. Bausch-Jurken MT, Verbsky JW, Gonzaga KA, Elms NP, Hintermeyer MK, Gauld SB, et al. The Use of Salmonella Typhim Vaccine to Diagnose Antibody Deficiency. J Clin Immunol. 2017 Jul;37(5):427–33.
65. Evans C, Bateman E, Steven R, Ponsford M, Cullinane A, Shenton C, et al. Measurement of Typhi Vi antibodies can be used to assess adaptive immunity in patients with immunodeficiency. Clin Exp Immunol. 2018 Jun;192(3):292–301.
66. Ochoa-Grullón J, Benavente Cuesta C, Pérez López C, Peña Cortijo A, Rodríguez de la Peña A, Álvarez Carmona A, et al. Evaluation of Polysaccharide Typhim Vi Antibody Response as a predictor of Humoral Immunodeficiency in Haematological Malignancies. Clin Immunol. 2020 Jan;210:108307.
67. Abolhassani H, Aghamohammadi A, Fang M, Rezaei N, Jiang C, Liu X, et al. Clinical implications of systematic phenotyping and exome sequencing in patients with primary antibody deficiency. Genet Med. 2019 Jan;21(1):243–51.
68. Park JH, Resnick ES, Cunningham-Rundles C. Perspectives on common variable immune deficiency. Ann N Y Acad Sci. 2011 Dec;1246:41–9.
69. Thaventhiran JED, Lango Allen H, Burren OS, Rae W, Greene D, Staples E, et al. Whole-genome sequencing of a sporadic primary immunodeficiency cohort. Nature. 2020 Jul;583(7814):90–5.
70. Tuijnenburg P, Lango Allen H, Burns SO, Greene D, Jansen MH, Staples E, et al. Loss-of-function nuclear factor κB subunit 1 (NFKB1) variants are the most common monogenic cause of common variable immunodeficiency in Europeans. J Allergy Clin Immunol. 2018 Oct;142(4):1285–96.

71. Lorenzini T, Fliegauf M, Klammer N, Frede N, Proietti M, Bulashevska A, et al. Characterization of the clinical and immunologic phenotype and management of 157 individuals with 56 distinct heterozygous NFKB1 mutations. J Allergy Clin Immunol. 2020 Oct;146(4):901–11.
72. Salzer U, Bacchelli C, Buckridge S, Pan-Hammarström Q, Jennings S, Lougaris V, et al. Relevance of biallelic versus monoallelic TNFRSF13B mutations in distinguishing disease-causing from risk-increasing TNFRSF13B variants in antibody deficiency syndromes. Blood. 2009 Feb;113(9):1967–76.
73. Speletas M, Mamara A, Papadopoulou-Alataki E, Iordanakis G, Liadaki K, Bardaka F, et al. TNFRSF13B/TACI alterations in Greek patients with antibody deficiencies. J Clin Immunol. 2011 Aug;31(4):550–9.
74. Rachid R, Castigli E, Geha RS, Bonilla FA. TACI mutation in common variable immunodeficiency and IgA deficiency. Curr Allergy Asthma Rep. 2006 Sep;6(5):357–62.
75. Lopez-Herrera G, Tampella G, Pan-Hammarström Q, Herholz P, Trujillo-Vargas CM, Phadwal K, et al. Deleterious mutations in LRBA are associated with a syndrome of immune deficiency and autoimmunity. Am J Hum Genet. 2012 Jun;90(6):986–1001.
76. Schubert D, Bode C, Kenefeck R, Hou TZ, Wing JB, Kennedy A, et al. Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. Nat Med. 2014 Dec;20(12):1410–6.
77. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110δ result in T cell senescence and human immunodeficiency. Nat Immunol. 2014 Jan;15(1):88–97.
78. van Schouwenburg PA, Davenport EE, Kienzler A-K, Marwah I, Wright B, Lucas M, et al. Application of whole genome and RNA sequencing to investigate the genomic landscape of common variable immunodeficiency disorders. Clin Immunol. 2015 Oct;160(2):301–14.
79. Rodríguez-Cortez VC, Del Pino-Molina L, Rodríguez-Ubreva J, Ciudad L, Gómez-Cabrero D, Company C, et al. Monozygotic twins discordant for common variable immunodeficiency reveal impaired DNA demethylation during naïve-to-memory B-cell transition. Nat Commun. 2015 Jun;6:7335.
80. Rae W. Indications to Epigenetic Dysfunction in the Pathogenesis of Common Variable Immunodeficiency. Arch Immunol Ther Exp (Warsz). 2017 Apr;65(2):101–10.
81. Heo JB, Lee Y-S, Sung S. Epigenetic regulation by long noncoding RNAs in plants. Chromosom Res an Int J Mol Supramol Evol Asp Chromosom Biol. 2013 Dec;21(6–7):685–93.
82. Bogaert DJA, De Bruyne M, Debacker V, Depuydt P, De Preter K, Bonroy C, et al. The immunophenotypic fingerprint of patients with primary antibody deficiencies is partially present in their asymptomatic first-degree relatives. Haematologica. 2017 Jan;102(1):192–202.
83. Driessen GJ, van Zelm MC, van Hagen PM, Hartwig NG, Trip M, Warris A, et al. B-cell replication history and somatic hypermutation status identify distinct pathophysiologic backgrounds in common variable immunodeficiency. Blood. 2011 Dec;118(26):6814–23.
84. Schatorje EJH, Gathmann B, van Hout RWNM, de Vries E. The PedPAD study: boys predominate in the hypogammaglobulinaemia registry of the ESID online database. Clin Exp Immunol. 2014 Jun;176(3):387–93.
85. El-Helou SM, Biegner A-K, Bode S, Ehl SR, Heeg M, Maccari ME, et al. The German National Registry of Primary Immunodeficiencies (2012-2017). Front Immunol. 2019;10:1272.
86. Odnoletkova I, Kindle G, Quinti I, Grimbacher B, Knerr V, Gathmann B, et al. The burden of common variable immunodeficiency disorders: a retrospective analysis of the European Society for Immunodeficiency (ESID) registry data. Orphanet J Rare Dis. 2018 Nov;13(1):201.
87. Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. Front Neuroendocrinol. 2014 Aug;35(3):347–69.
88. LEDLEY RS, LUSTED LB. The role of computers in medical diagnosis. Med Dok. 1961 Jul;5:70–8.
89. Gorry GA, Barnett GO. Sequential diagnosis by computer. JAMA. 1968 Sep;205(12):849–54.

90. Miller RA. Medical diagnostic decision support systems--past, present, and future: a threaded bibliography and brief commentary. J Am Med Inform Assoc. 1994;1(1):8–27.
91. Miller RA. Computer-assisted diagnostic decision support: history, challenges, and possible paths forward. Adv Health Sci Educ Theory Pract. 2009 Sep;14 Suppl 1:89–106.
92. Barnett GO, Famiglietti KT, Kim RJ, Hoffer EP, Feldman MJ. DXplain on the Internet. Proceedings AMIA Symp. 1998;607–11.
93. Berger SA. GIDEON: a computer program for diagnosis, simulation, and informatics in the fields of geographic medicine and emerging diseases. Emerg Infect Dis. 2001;7(3 Suppl):550.
94. Vardell E, Moore M. Isabel, a clinical decision support system. Med Ref Serv Q. 2011;30(2):158–66.
95. Riches N, Panagioti M, Alam R, Cheraghi-Sohi S, Campbell S, Esmail A, et al. The Effectiveness of Electronic Differential Diagnoses (DDX) Generators: A Systematic Review and Meta-Analysis. PLoS One. 2016;11(3):e0148991.
96. Weber GM, Mandl KD, Kohane IS. Finding the missing link for big biomedical data. JAMA. 2014 Jun;311(24):2479–80.
97. Chen K, Coonrod EM, Kumánovics A, Franks ZF, Durtschi JD, Margraf RL, et al. Germline mutations in NFKB2 implicate the noncanonical NF-κB pathway in the pathogenesis of common variable immunodeficiency. Am J Hum Genet. 2013 Nov;93(5):812–24.
98. Sun S-C. The non-canonical NF-κB pathway in immunity and inflammation. Nat Rev Immunol. 2017 Sep;17(9):545–58.

PART III

Addendum

Summary

SUMMARY

Ear-nose-throat (ENT) and lower airway symptoms commonly occur, which are usually innocent and self-limiting. When infections continuously recur, this can be a sign of underlying primary immunodeficiency (PID). Of all PIDS, hypogammaglobulinaemias form the largest group, but they are still very rare. Most health care professionals, who are not specialised in immunodeficiency, do not consider potential PID in patients with common symptoms, such as recurrent "normal" infections and chronic fatigue. These patients are therefore often diagnosed late, particularly those with milder phenotypes presenting at later stages. As a consequence, they suffer long uncertainty, multiple hospital attendances, investigations, misdiagnoses, and inappropriate treatments, resulting in huge emotional cost and wasted time, effort and resources. Reducing diagnostic delay is therefore important. In this thesis, clinical presentation patterns of single- and multicentre patient cohorts with hypogammaglobulinaemia and easier-to-interpret diagnostic tests are presented and discussed in detail with the aim to improve earlier detection of hypogammaglobulinaemia.

Chapter 1 is an introduction to the general principles of immunity, underlying genetic defects and clinical presentation of hypogammaglobulinaemia, with a special emphasis on unclassified primary antibody deficiencies (unPAD), including the problem of diagnostic delay and explanation why we set up the unPAD study. Subsequently, an outline of the content is given and the aims of this thesis are described:

1. To describe a secondary centre cohort with primary antibody deficiency (PAD), in whom the majority has unPAD.
2. To review all previously published patients with decreased serum IgM and describe a cohort of Dutch patients with persistent, isolated decreased serum IgM.
3. To describe a larger multicentre European cohort of patients with IgM deficiency using data of the ESID Online Database.
4. To review all existing data on the clinical presentation and follow-up of common variable immune deficiency (CVID).
5. To present the design and rationale for the unPAD study.
6. To explore the journey to a PAD diagnosis from the perspective of patients and to analyse how these patients appraise their symptoms and which factors are involved in a decision to seek medical care.
7. To investigate the application of the 23-valent anti-PnPS IgG assay for predicting good responders to Pneumococcal polysaccharide vaccination in a general hospital population setting.
8. To investigate the clinical relevance of 23-valent anti-PnPS IgM and IgA assays in addition to the anti-PnPS IgG assay.

Chapter 2 gains insight into the clinical characteristics and health-related quality of life (HRQoL) of patients with PAD, mainly unPAD. Data from 23 children and 99 adults with PAD, collected as part of a standardized, 1-day Care Pathway for suspected primary immunodeficiency, were analysed. This study shows that unPAD can result in a severe patient burden, implicating that "mild" hypogammaglobulinaemia can be a serious condition. A high proportion of unPAD patients suffered from bronchiectasis and their HRQoL was significantly impaired in all domains compared to the reference population. This means that a lot of unPAD patients had to cope simultaneously with pain, negative feelings and impairments in cognition, home management tasks, sleep, social interaction, and work. The most prominently impaired HRQoL domain was vitality, indicating these patients feel extremely tired and worn out. This study highlights the need for the clinician to acknowledge the existence of unPAD and be aware of its potential consequences, in order to timely and appropriately manage its effects and complications.

In **chapter 3**, we describe all previously published patients with decreased serum IgM and a cohort of Dutch patients with persistent, isolated decreased serum IgM in a large teaching hospital in 's-Hertogenbosch, the Netherlands. Selective IgM deficiency (sIgMdef) is defined as a serum IgM level repeatedly below 2 standard deviations of normal with normal levels of serum IgA, IgG and IgG-subclasses, normal vaccination responses, absence of T cell defects and absence of causative external factors. Our main finding was that true sIgMdef is probably very rare. Unfortunately, when a decreased serum IgM level is found, it is rarely analysed fully: data on IgG subclasses and/or vaccination responses were lacking in 74% literature cases and 93% of cases from our cohort. Also, different criteria for 'selective IgM deficiency' are used in the literature; in a quarter of literature cases the deficiency was not 'selective', other immunological abnormalities were present. Only 2% of literature cases and 7% of cases from our cohort completely fulfilled the ESID criteria. These results illustrate the clinical challenge of determining the relevance of a serum sample with decreased serum IgM.

Chapter 4 shows the results of the SIMcal study: a registry study using the ESID online database. Characteristics from 98 patients with decreased serum IgM collected in 12 countries are described. When isolated decreased serum IgM levels are repeatedly found in a patient, clinicians are confronted with a dilemma. To date it is not clear what the clinical consequences of such a finding are, and whether and if so how such patients should be treated. Even this multi-centre study could not solve this dilemma. Only ten patients completely fulfilled the ESID criteria for true sIgMdef, and when using the ESID diagnostic protocol reference values, only six patients had true sIgMdef (age-matched cut-off values varied widely between centres). Because of these small numbers, further analyses were performed in patients with true or possible sIgMdef (13 adults, 48 children). Adults with more severely decreased serum IgM levels were more likely to be younger and to be symptomatic. This information can

help in interpreting the clinical significance when an isolated decreased serum IgM level is discovered. If we want to explore its clinical consequences, we should fully analyse and accurately describe those patients in whom a decreased serum IgM level is found.

Chapter 5 systematically reviews all existing data on the clinical presentation and follow-up of common variable immune deficiency (CVID). Our meta-analysis of 51 studies (n=8512 patients), identifying 134 presenting and 270 total clinical manifestations, confirmed the high frequency of respiratory infections at presentation (75%), but also showed a high incidence of severe bacterial infections such as sepsis (8%) and meningitis (6%), and immune dysregulation features including bronchiectasis (28%), lymphadenopathy (27%), splenomegaly (13%), inflammatory bowel disease (11%), autoimmune cytopenia (10%) and idiopathic thrombocytopenia (6%). These findings can help clinicians to recognise CVID, and to estimate how common a clinical manifestation is in paediatric and adult CVID. The data showed clear differences in clinical manifestations occurring during the disease course between children and adults with CVID, with more non-infectious disease complications in adults. This implies that different monitoring strategies are warranted for children and adults during follow-up. This study also revealed a bimodal sex distribution, with male predominance in children (62%) and female predominance in adults (58%), which suggests differences in genetic and environmental aetiology in CVID between adults and children and has consequences for pathophysiologic studies.

Chapter 6 presents the design and rationale for 'the unPAD study'. Unclassified antibody deficiencies (unPADs) are characterised by decreased total IgG, IgG-subclass(es), IgM, IgA and/or specific antibodies, alone, or in combinations, but not fulfilling the criteria for CVID. They are considered milder forms of hypogammaglobulinemia and are therefore often ignored in clinical practice and the literature. UnPADs often remain undiagnosed for years, but can ultimately lead to important morbidity, irreversible organ damage, and loss of lifespan when they are not recognized and adequately treated. Therefore, we designed the unPAD study to describe in detail all types of PAD patients without a known specific monogenetic origin (thus excluding e.g. X-linked and autosomal recessive agammaglobulinemia, and class-switch recombination defects) regarding their clinical and immunological pattern at presentation and during follow-up using the ESID online Registry. Because clinical severity as well as the results of immunological laboratory investigations and potential underlying pathophysiology may differ greatly within this group, this study also aims to identify subgroups based upon these clinical and immunological characteristics. All data will be monitored and – if necessary – corrected before statistical exploration of the registered data will be performed. This study is currently running, and 10 participating centres have already been monitored. Many centres are still registering their patient data into the ESID online Database and will be monitored in the future.

Chapter 7 focusses on the journey to a PAD diagnosis from the perspective of patients. This qualitative study revealed presenting patterns that can help identify those patients who are 'always ill' and 'worn out' with PAD and factors that are involved in a decision to seek medical care. Remarkably, PAD patients tended to normalise their symptoms and carry on with usual activities. Medical presenting patterns included: 1) infections being unusually frequent and/or severe, not clearly season-bound, requiring antibiotics to clear, and 2) undergoing tympanoplasty, sinus surgery and/or polypectomy. Factors negatively affecting the speed and accuracy of diagnosing PAD included: 1) misattributing the presenting signs and symptoms to common, self-limiting illnesses, 2) lack of knowledge about the clinical presentation of PAD in general practitioners and non-immunologists, 3) reluctance to seek care because of getting so used to symptoms that patients therefore considered to be normal, feelings of not being taken seriously and a negative quality of the doctor-patient relationship. This study underlines the importance of education programmes, which should not only focus on the medical 'red flags' of PID, but also on coping strategies of more common, less differentiating symptoms such as 'being always ill' and 'worn out'. However, education programmes alone cannot be the final solution, because it is impossible for non-experts to know about all >8000 rare diseases. Hopefully, modern developments in automated pattern recognition can be developed to offer 'red flags' in the electronic patient file that alert a physician to potential underlying problems. This qualitative analysis can help the design of predictive models in this regard.

Chapter 8 reveals a new-developed decision tree, showing that the 23-valent Pneumococcal IgG assay can be a reliable screening test to predict good responders to PnPS-vaccination in conjugated-vaccine-naïve patients in a low pre-test probability setting, with the serotype-specific assay as a second-line test. All patients with a pre-immunization-titre ≥38.2 µg/ml and/or post-immunization-titre ≥ 96.1µg/ml and none with a post-immunization-titre ≤ 38.5µg/ml exhibited a good response to PnPS vaccination. Only 24% of patients would require further serotyping when these breakpoints as screening test to predict good responders are used. This analysis supports implementation of the 23-valent IgG assay to lower the threshold for timely detection of PAD by reducing the overall number of patient samples needing serotype specific antibody measurement, thus reducing overall costs.

Chapter 9 reports the results of anti-PnPS IgM and IgA assays in addition to anti-PnPS IgG assays (240 patients; 304 samples) in patients with potential PAD in a general hospital population. This study revealed that patients classified as having an intact antibody response based on the measurement of serotype-specific PnPS IgG, still can display impaired anti-PnPS IgM and IgA responses. Because none of the patients with a decreased anti-PnPS IgM or IgA response had been classified as 'no PAD' based on serotype-specific PnPS IgG testing and serum immunoglobulin levels, the clinical relevance of 'specific IgA or IgM antibody deficiency' could not be determined based on our data. Adding anti-PnPS IgA and IgM assays

to the anti-PnPS IgG assay could not reduce the need for the more expensive and difficult to interpret serotype specific PnPS IgG testing. Therefore, based on our data, it does not seem useful for a clinician in a general hospital to request anti-PnPS IgA and IgM assays in addition to the anti-PnPS IgG assay.

Chapter 10 provides a general discussion, in which the main findings of this thesis are discussed, followed by implications and directions for further research. Taken together, patients with 'milder' forms of hypogammaglobulinaemia, i.e. unclassified primary antibody deficiencies (unPAD), are often diagnosed late, ignored in clinical practice and the literature, and incompletely analysed nor accurately described. Also for these patients, early detection and adequate treatment is important, because unPAD can be a serious condition; 44% of patients in our cohort already showed bronchiectasis at presentation and their health-related quality of life was significantly decreased in all domains. Our meta-analysis reveals that an exclusive focus on the currently existing infection-centred warning signs would miss around 25% of CVID patients that initially present with immune dysregulation features. In addition to these medical aspects, non-medical aspects, such as coping strategies of patients who are 'always ill' and 'worn out', can help to distinguish hypogammaglobinaemia from chronic fatigue syndrome or innocent and self-limiting infections. Our developed decision tree, using the 23-valent Pneumococcal IgG assay as a first-line test and the serotype-specific assay as a second-line test, can be used as a reliable screening tool for earlier detection of hypogammaglobinaemia. In order to confirm our results and further investigate clinical presentation patterns and complications of unPAD patients, a larger patient cohort is necessary. This could also enable the identification of potential subgroups, identifying which patients are clinically more severe, and need strict follow-up and potential different treatment strategies. For this reason, we developed the unPAD study, a multi-centre registry study based on the ESID online database, which is still ongoing.

Dankwoord

DANKWOORD

Het bijzondere moment is gekomen dat dit proefschrift bijna klaar is. Zonder de inzet en steun van vele enthousiaste en bereidwillige mensen, was dit nooit gelukt. Hieronder wil ik iedereen bedanken, zonder wiens inspanningen en steun dit proefschrift niet tot stand zou zijn gekomen.

Allereerst wil ik alle kinderen (en ouders) en volwassenen bedanken die hebben deelgenomen aan de verschillende onderzoeken. Dank voor alle patiënten die toestemming gaven voor registratie van hun data in het ESID register. Dank voor alle patiënten die het zorgpad immunologie bezochten in het Jeroen Bosch Ziekenhuis, 's-Hertogenbosch en toestemming gaven voor afname van extra serumbuizen en gebruik van hun data. Dank voor alle deelnemers die zich bereidwillig stelden zich te laten interviewen en ons zo zeer waardevolle inzichten gaven in alle aspecten naar de weg naar de diagnose van antistof deficiëntie. Dankzij jullie gaat het lukken om stappen vooruit te zetten in de herkenning, karakterisering, behandeling en subclassificering van primaire antistof deficiënties.

Prof. dr. E. de Vries, beste Esther,
Wat begon als een schakeljaar kindergeneeskunde in 's-Hertogenbosch, eindigt hier met een proefschrift. Jij zag een talent in mij, waar ik in het begin zelf niet van overtuigd was en jij gaf mij de mogelijkheid om me hierin verder te ontwikkelen. Stap voor stap, met heldere opbouwende feedback, leerde je me analyseren, kritisch reflecteren, structureren en sterke, korte boodschappen overbrengen. Jij was voor mij de beste leerschool en ik wil je daar echt ontzettend voor bedanken. Door je immer snelle feedback – die altijd concreet was – was het mogelijk om vaart te houden en alle onderzoeksprojecten te combineren met mijn opleiding tot kinderarts. We hebben samen hard gewerkt om de unPAD studie op de kaart te zetten. Ik weet zeker dat het je gaat lukken om heel waardevolle data uit deze studie te verkrijgen die de levens van patiënten met een primaire antistof deficiëntie in positieve zin gaat beïnvloeden. Heel dankbaar ben ik je voor alle congressen, cursussen en summer schools waarop je me attendeerde, en waar je me ook altijd de mogelijkheid tot deelname voor gaf. Enorm bedankt voor je begeleiding de afgelopen jaren!

Dr. M. van der Flier, beste Michiel,
Wat een geweldig moment was het toen jij als copromotor betrokken werd bij mijn traject. Je was een grote aanwinst voor mijn promotieteam. Je schoof vlekkeloos aan bij het al lopende traject en wist een waardevolle bijdrage te geven aan dit proefschrift. Ik bewonder jouw talent om data te vertalen naar praktisch toepasbare conclusies en nooit de hoofdboodschappen uit het oog te verliezen. Dankjewel dat ik veel van je heb mogen leren!

Hierbij wil ik de leden van de manuscriptcommissie, prof. dr. A.A. de Bont, prof. dr. X. Bossuyt, prof. dr. G.J.A. Driessen, dr. V.A.S.H. Dalm, dr. C. Obihara, bedanken voor de beoordeling van mijn manuscript.

Ook wil ik alle coauteurs van alle artikelen heel hartelijk bedanken voor hun hulp en commentaren.

Extra dank aan Ineke Reijnen, zonder jou was het schrijven van het unPAD protocol artikel nooit zo snel gegaan. Ik bewonder hoe je je kwetsbaar durft op te stellen en op latere leeftijd aan een nieuw traject durft te gaan beginnen. Jij laat zien dat je nooit te oud bent om te leren. Ik weet zeker dat je de 'unPAD study' een krachtig vervolg gaat geven. Je weet me te vinden in de toekomst!

Roeland van Hout, ik wil je bedanken voor je hulp bij de statische analyses van de 'SIMcal study' en het meedenken over de statiek van de 'unPAD study'. Jouw inzichten maakten de statistische analyses waterdicht. Zelfs de reviewers hebben er veel van geleerd.

Paul Basset, ook jou wil ik bedanken voor je hulp bij de statische analyses. Ook al was – naar later bleek - de groep antistof deficiënte patiënten te klein voor subgroep analyses, dit gaf belangrijke inzichten in welke statistiek ons hierin op dit vlak verder kon brengen. Deze inzichten gaan ons helpen bij de data-analyses van de 'unPAD study'.

Dear Benjamin Gathmann, thank you for your ever-fast correspondence regarding my questions about data insertion and extraction from the ESID Online Database. I will miss your coded emails, fast reply's and reliability.

Graag wil ik de kinderarts-immunologen en internist-immunologen uit de verschillende ziekenhuizen in Nederland, België, Duitsland, Slowakije, Italië, Tsjechië, Spanje, Zwitserland, Griekenland, Verenigd Koninkrijk en Turkije bedanken voor de invoer van hun patiënt data in het ESID register. Speciale dank wil ik uitspreken aan Marina Garcia en Pere Soler Palacin uit Spanje, Leif Hanitsch en Renate Krüger uit Duitsland, Maria Carrabba en Giovanna Fabio uit Italië, Lucia Baselli en Rosa Dellepiane uit Italië, Efimia Papadopoulou-Alataki en Kiki Chiorna uit Griekenland, Milos Jesenak, Lenka Kapustova en Otilja Petrovic uit Slowakije, Bram Rutgers, Annick van de Ven, Stefanie Henriet, Koen van Aerde, Jaap ten Oever, Anna Simon, Riet Strik, Judith Potjewijd en Suzanne Bazen uit Nederland, voor het hartelijk ontvangen van mij tijdens mijn monitor visites. Jullie warme welkom en betrokkenheid heeft niet alleen gezorgd voor waardevolle data, maar ook voor mooie mogelijke nieuwe samenwerkingsverbanden in de toekomst.

Dr. M. Heron, beste Michiel, ik wil je bedanken voor je inzet bij het verrichten van de 23-valente IgG assays en serotype-specifieke pneumococcen polysacchariden assays en je hulp bij de analyses hiervan. De verschillende typen samples maakten de data-analyses heel uitdagend. Zonder jouw hulp was dit me nooit gelukt! Jean-Luc Murk, jij nam de functie van Michiel Heron over. Ik wil je bedanken voor je altijd zeer enthousiaste reacties op mijn mails en positieve feedback op mijn artikelen. Daarna heb je je ingezet voor het verrichten van 23-valente IgA en IgM assays, bedankt daarvoor!

Prof dr. G. Rijkers, Ger, wat beschik jij over een ongelofelijk arsenaal aan kennis over alles wat met de bacterie *Streptococcus pneumoniae* te maken heeft. Niet alleen gaf jij belangrijke inzichten bij het schrijven van de artikelen over de pneumococcen polysacchariden assays, ook zorgde jij ervoor dat we de reviewers fluitend konden beantwoorden. Zonder jou waren deze artikelen nooit zo snel gepubliceerd.

Dr. A.C.A.P. Leenders, beste Alexander, bedankt voor je inzet bij de analyses van de in het Jeroen Bosch Ziekenhuis verzamelde patiënten serumbuizen. Ik kijk ernaar uit om nu ook zelf aan de slag te gaan als kinderarts in het Jeroen Bosch Ziekenhuis en vind het fijn dat jij deel uitmaakt van de wekelijkse 'dinsdag besprekingen'.

Beste medewerkers van The Binding Site Group, specifiek Markus Skold en Antony Parker, bedankt voor de donatie van de pneumococcen serologie kits. Extra dank aan Markus, jouw gave om data duidelijk visueel weer te geven is bijzonder. Bedankt dat je je expertise op dit vlak met ons wilde delen.

Prof. dr. J. de Vries, Jolanda, door jouw expertise op het vlak van 'kwaliteit van leven studies' hebben we onze data analyses naar een hoger niveau kunnen tillen. Nog niet eerder was de kwaliteit van leven van patiënten met een ongeclassificeerde primaire antistof deficiëntie zo nauwkeurig onderzocht. Dankjewel voor je goede ideeën hierover.

Medisch specialisten van het Jeroen Bosch Ziekenhuis, internist-pulmonoloog drs. Thomas Macken, internist-oncoloog dr. Hans Pruijt, internist-reumatoloog dr. Marjonne Creemers en neuroloog dr. Jeroen van Eijk. Bedankt voor het aanleveren van data van de IgM-deficiënte patiënten uit het Jeroen Bosch Ziekenhuis en het vervolgens reviseren van dit artikel. Het was bovendien erg leuk om een keer de niet-kindergeneeskundige kant van het Jeroen Bosch Ziekenhuis te zien. Dr. Arnoud Knoops, bedankt voor je nauwkeurige beoordeling van de HRCT-scans. Ik kijk uit naar meer samenwerkingsverbanden in de toekomst.

Natuurlijk wil ik ook alle collega's van de afgelopen jaren bedanken voor de prettige samenwerking. Alle arts-assistenten in het Jeroen Bosch Ziekenhuis en het Radboudumc tijdens mijn opleidingstijd. Het waren ontzettend fijne tijden!

Beste Ellen, ik heb me altijd vereerd gevoeld om jouw opvolger te mogen zijn. Je was er altijd voor me als ik vragen had en nam er uitgebreid de tijd voor. Ik leerde je nog beter kennen tijdens mijn verdiepingsstage kinderreumatologie. Ik heb veel bewondering voor je diepgaande kennis over niet alleen de kinderreumatologie, maar ook de algemene kindergeneeskunde, en hoe je altijd de rust weet te bewaren ondanks drukte en dit zelfs nog weet te combineren met een gezin. Je bent voor mij een inspirator en voorbeeld. Ik hoop dat we nog veel met elkaar mogen samenwerken in de toekomst; de woon afstand is in ieder geval al kleiner geworden.

Mede-PhD studenten en onderzoekers aan Tranzo. De woensdagen samen waren iets om naar uit te kijken. Door de grote diversiteit aan studie achtergronden, hebben we veel van elkaar geleerd op verrassende gebieden. Waar kwalitatief onderzoek voor mij nog een onbekend terrein was, hielpen jullie me op weg. Vincent, jij was de eerste die de lang verwachte woorden 'Hora est' hoorde, bedankt voor je hulp bij de praktische kant van dit traject. Francine, Noortje, Vera en Evita, bedankt voor de fijne samenwerking, de gezellige lunches en veel succes met de afronding van jullie PhD projecten.

Extra dank aan de kinderartsen in het Jeroen Bosch Ziekenhuis voor de tijd en kansen die jullie mij hebben gegeven om onderzoek met mijn opleiding tot kinderarts te combineren. Ik voel me vereerd dat ik zometeen ook als kinderarts aan de slag mag gaan in dit ziekenhuis. Ik kijk ontzettend uit naar mijn toekomst binnen jullie team, het heeft altijd gevoeld als een warm bad!

Dr. E. Coolen en Dr. J. van der Velden, beste Ester en Janielle, dankjulliewel voor jullie prettige opleidingsrol. Ik heb me thuis gevoeld onder jullie hoede en heb me altijd ontzettend gesteund gevoeld. Zonder de tijd die jullie me gaven om te schrijven en jullie bemoedigende woorden, was het me niet gelukt om dit proefschrift zo snel af te krijgen.

Dr. J. Draaisma en dr. J. Geelen, beste Jos en Joyce, kinderartsen-EAA, jullie gaven mij de mogelijkheid het vak EAA van jullie te leren. Ik voel me trots dat ik de bij jullie opgedane kennis in de praktijk mag gaan uitdragen in het Jeroen Bosch Ziekenhuis. Heel dankbaar ben ik voor alles wat ik van jullie geleerd heb.

De afgelopen 2 jaar heb ik het geluk gehad om mee te mogen doen met het TULIPS-PhD curriculum. Lieve Anne, Tim, Lisa, Elise, Fleur, Kelly, Maud, Victoria, Jessica, Yvette, Hanneke, Marijn, Anne-Fleur, Jenneke, Myrthe, Nicole, Josine en Emma, wat hebben we fijne, leerzame TULIPS dagen en weekenden beleefd. Ik hoop dat we elkaar blijven vinden in de toekomst, ik kijk ernaar uit!

Graag wil ik ook al mijn lieve vrienden en familie bedanken.
Jaargenootjes/ vriendinnen, lieve Ingeborg en Stefanie, wat ben ik dankbaar dat jullie aan mijn zijde hebben gestaan de afgelopen 5 jaar. Gelukkig niet alleen tijdens de afgelopen opleidingsjaren, maar ongetwijfeld nog veel jaren hierna. Bedankt voor de talloze eet avondjes, koffie momenten op werk en dat we partners in crime waren in de sociale commissie. Ik vind het ontzettend bijzonder dat jullie ook vandaag naast me staan als paranimfen. Co-groep genootje Merel, wat ben ik blij dat we samen in dezelfde co-groep terecht kwamen. Een bijzondere vriendschap ontstond. Samen konden we elkaar steunen in een gelijkwaardig traject wat we samen doorliepen; jij als neuroloog in opleiding en ik als kinderarts in opleiding. Mijn lieve Liemerse Lummels en Lady's, Marlis, Alieke, Femke, Angela, Ieke, Jan Willem en Sander, dankjewel voor al jullie gezelligheid de afgelopen jaren. Ik kijk altijd enorm uit naar onze weekenden samen, het kan niet gek genoeg. Onze vriendschap heeft een bijzondere basis, gestart tijdens de middelbare schoolperiode, en zal ondanks de fysieke afstand tussen elkaar, hopelijk altijd stand blijven houden.

Lieve Vera, ik ben ontzettend blij dat we tijdens bij de start van de opleiding geneeskunde in dezelfde werkgroep ingedeeld. We wisten allebei gelijk dat het klopte en dit is ook zo gebleken. Ik bewonder hoe je je eigen weg durfde in te slaan, die anders is dan die van veel studiegenoten. Jij durft 'out-of-the-box' te gaan en zo te kiezen voor je eigen geluk. Ik ben dankbaar voor de bijzondere band die we hebben ontwikkeld. We hebben lief en leed met elkaar gedeeld. Hoeveel stappen zouden wij samen gezet hebben? Te veel om te tellen, evenals onze momenten samen. Lieve Veer, blijf zoals je bent!

Lieve Pauline, wat ben ik ontzettend dankbaar dat je op 4-jarige leeftijd bij mij in de straat kwam wonen. Een bijzondere, tijdloze vriendschap ontstond en werd nog hechter tijdens onze afgelopen Nijmeegse jaren. Ik bewonder je doorzettingsvermogen, je positieve houding, je immense hoeveelheid energie en kracht. Ik ben ontzettend dankbaar voor je steun tijdens de emotionele uitdagingen die het leven biedt. Met mijn verhuizing naar 's-Hertogenbosch zullen onze meer wekelijkse hardlooprondjes niet meer zo vanzelfsprekend zijn, wat ga ik dit missen! Ik weet dat wij altijd verbonden zullen blijven.

Lieve opa Henk, oftewel, Hendrik de vierde van Pirandello, nog niet zo lang geleden nam je afscheid. Ik zal nooit vergeten hoe je de centen altijd in de rondte gooide. Lieve opa Wim, ook jij hebt inmiddels afscheid genomen. Wat ben ik dankbaar dat je nog op onze bruiloft kon zijn. Lieve oma Ria, jij volgde niet veel later, maar verliet het leven met een lach, welke me altijd zal bijblijven. Lieve oma Marianne, wat prijs ik mezelf gelukkig dat ik zelfs op deze leeftijd nog een oma heb, en wat voor een! Je sprankelt nog steeds, al is het leven niet makkelijk zonder opa.

Lieve Steef en Willy, heel erg bedankt voor jullie interesse en support de afgelopen jaren. Ik voel me enorm door jullie gesteund en weet dat jullie er altijd voor ons zullen zijn. Met jullie vieren we de pieken, en staan we stil bij de dalen. Hannelore, ook jou wil ik niet ongenoemd laten. Door jou hier op papier te zetten, ben je er toch nog bij. We missen je en houden ontzettend veel van je. Dankjewel voor je onvoorwaardelijke liefde al die jaren. Weet dat we deze liefde altijd voor jou zullen houden en er altijd bij stil zullen blijven staan.

Lieve Tim en Emmy, wat hebben we een fijne jeugd samen gehad. Jullie zijn beiden ouders van prachtige kinderen. Een trotsere peettante kunnen ze zich niet wensen.

Lieve papa en mama, hoe moet ik in woorden omschrijven hoe dankbaar ik jullie ben? Ik kan me geen meer toegewijde ouders wensen. Papa, oftewel 'dad', we hebben altijd een bijzondere band gehad. Jouw vermogen om anderen echt te begrijpen is een gave, waar je al veel anderen mee geholpen hebt. Ik heb er bewondering voor hoe je met je humor ook de minder leuke kanten van het leven leuk maakt. Ik ben dankbaar dat ik jouw rust en discipline heb geërfd. Boven alles dankjewel voor je onvoorwaardelijke steun en liefde. Mama, jij knapt werkelijk uit elkaar van liefde voor je kinderen. Het is niet voor te stellen hoe je er altijd voor mij bent geweest. Van jou heb ik mijn doorzettingsvermogen en perfectionisme, onmisbare eigenschappen, maar ook mijn grootste valkuilen, jou maar al te goed bekend. Mijn zorgen werden jouw zorgen, maar daar tegenover werd mijn geluk ook jouw geluk. Dankjewel voor je onvoorwaardelijke liefde. Deze woorden heb je eigenlijk niet nodig, onze telepathie is voldoende.

Tenslotte, het beste heb ik bewaard tot het laatste. Mijn allerbelangrijkste, lieve Wout. Je bent al meer dan 12 jaar het allerbeste wat me is overkomen! Je wist hoe belangrijk dit traject voor me was, en hebt me er altijd in gesteund. Jij maakt alles in het leven lichter, met jou ben ik heel gelukkig!

Curriculum Vitae

CURRICULUM VITAE

Lisanne Marianne Antoinette Janssen, dochter van Ingrid Catharina Theodora Janssen-Berendsen en Koen Henricus Wilhelmus Janssen, werd geboren op 7 december 1990 in Duiven. Daar groeide ze op samen met haar broer Tim en zusje Emmy. Na het behalen van haar gymnasiumdiploma (cum laude) op het Liemers college in Zevenaar is zij in 2009 gestart met de studie geneeskunde aan de Radboud universiteit in Nijmegen. Tijdens de geneeskunde opleiding schreef zij de Reader Farmacotherapie onder supervisie van Prof. Dr. C. Kramers, wat gebruikt wordt als lesmateriaal voor studenten van alle Nederlandse geneeskunde faculteiten. Daarnaast nam zij deel aan de neurotop summer school in Nijmegen en gaf zij EHBO-cursussen aan eerste- en tweedejaars geneeskunde studenten. In 2015 werd ze aangenomen voor het dedicated schakeljaar kindergeneeskunde bij de neonatologie in het Radboudumc Amalia kinderziekenhuis en bij de algemene kindergeneeskunde in het Jeroen Bosch Ziekenhuis te 's-Hertogenbosch. Gedurende het schakeljaar heeft ze onder begeleiding van Dr. Liem onderzoek gedaan naar het effect van het gebruik van 0.2% chloorhexidine in acetaat als desinfectans op de patiëntveiligheid bij extreem premature neonaten. De resultaten hiervan mocht ze presenteren in 2015 op de PAS annual meeting in Baltimore, Verenigde Staten.

In 2016 behaalde ze haar artsexamen en aansluitend begon zij met haar specialisatie tot kinderarts. Haar perifere opleiding heeft ze gevolgd in het Jeroen Bosch Ziekenhuis te 's-Hertogenbosch onder leiding van prof. dr. E. de Vries en vervolgens dr. J.A.M. Widdershoven. Het academische deel van de opleiding tot kinderarts vond plaats in het Radboudumc Amalia kinderziekenhuis onder leiding van dr. J. van der Velden en dr. E. Coolen. Tijdens de opleiding is zij ook gestart met onderzoek naar het verbeteren van vroege herkenning van antistof deficiënties (in eerste instantie o.l.v. prof. dr. E. de Vries en later ook dr. M. van der Flier), wat uiteindelijk heeft geleid tot dit proefschrift. Gedurende haar promotie traject heeft Lisanne deelgenomen aan het TULIPS PhD curriculum.

De specialisatie tot kinderarts heeft zij in maart 2022 afgerond en zij is in mei 2022 gestart als algemeen kinderarts in het Jeroen Bosch Ziekenhuis. Lisanne is getrouwd met Wout Fontane Pennock en woont samen met hem in 's-Hertogenbosch.

Publications

PUBLICATIONS

Protocol for the unclassified primary antibody deficiency (unPAD) study: Characterization and classification of patients using the ESID online Registry.
Janssen LMA, Reijnen ICGM, Milito C, Edgar D, Chapel H, de Vries E, unPAD consortium.
PLoS One. 2022 Mar 25;17(3):e0266083.

Which triggers could support timely identification of primary antibody deficiency? A qualitative study using the patient perspective.
Janssen LMA, van den Akker K, Boussihmad MA, de Vries E.
Orphanet J Rare Dis. 2021 Jun 29;16(1):289.

The clinical relevance of IgM and IgA anti-pneumococcal polysaccharide ELISA assays in patients with suspected antibody deficiency.
Janssen LMA, Heron M, Murk JL, Leenders ACAP, Rijkers GT, de Vries E.
Clin Exp Immunol. 2021 Aug;205(2):213-221.

Lessons Learned From the Clinical Presentation of Common Variable Immunodeficiency Disorders: A Systematic Review and Meta-Analysis.
Janssen LMA, van der Flier M, de Vries E.
Front Immunol. 2021 Mar 23;12:620709.

Focusing on Good Responders to Pneumococcal Polysaccharide Vaccination in General Hospital Patients Suspected for Immunodeficiency. A Decision Tree Based on the 23-Valent Pneumococcal IgG Assay.
Janssen LMA, Heron M, Murk JL, Leenders ACAP, Rijkers GT, de Vries E.
Front Immunol. 2019 Nov 5;10:2496.

SIMcal Consortium. Challenges in investigating patients with isolated decreased serum IgM: The SIMcal study.
Janssen LMA, van Hout RWNM, de Vries E.
Scand J Immunol. 2019 Jun;89(6):e12763.

Mild Hypogammaglobulinemia Can Be a Serious Condition.
Janssen LMA, Bassett P, Macken T, van Esch J, Pruijt H, Knoops A, Sköld M, Parker A, de Vries J, de Vries E. Front Immunol. 2018 Oct 15;9:2384.

Truly selective primary IgM deficiency is probably very rare.
Janssen LMA, Macken T, Creemers MCW, Pruijt JFM, Eijk JJJ, de Vries E.
Clin Exp Immunol. 2018 Feb;191(2):203-211.

Reduction of chlorhexidine-induced chemical burns in extremely preterm infants by using 0.2% chlorhexidine-acetate as a skin disinfectant.
Janssen LMA, Tostmann A, Hopman J, Liem KD.
J Pediatr. 2018 Jun;197:319-320.

0.2% chlorhexidine acetate as skin disinfectant prevents skin lesions in extremely preterm infants: a preliminary report.
Janssen LMA, Tostmann A, Hopman J, Liem KD.
Arch Dis Child Fetal Neonatal Ed. 2018 Mar;103(2):F97-F100.

Abbreviations

ABBREVIATIONS

ANA	anti-nuclear antibodies
ANOVA	analysis of variance
AUC	area under the curve
BAFF-R	B cell activation factor receptor
BCR	B cell antigen receptor
BMI	body mass index
BTK	Bruton's kinase
CAML	calcium-modulating cyclophilin ligand
CASP	critical appraisal skills program
CD	cluster of differentiation
CI	confidence interval
COPD	chronic obstructive pulmonary disease
COREQ	consolidated criteria for reporting qualitative research
CPS	capsular polysaccharide
CSR	class-switch recombination
CTLA4	cytotoxic T-lymphocyte-associated 4
CVID	common variable immunodeficiency disorders
CWPS	cell wall polysaccharide
ESID	European Society for Immunodeficiencies
ENT	ear nose throat
ESID	European Society for Immunodeficiencies
GP	general practitioner
HIV	human immunodeficiency virus
HRCT	high resolution computerized tomography
HRQoL	health-related quality of life
HSC	hematopoietic stem cell
ICC	intraclass correlation coefficient
ICD	International Classification of Diseases
ICON	international consensus document
ICOS	inducible T cell costimulatory
Ig	immunoglobulin
IgGsc	IgG-subclass
ITP	idiopathic thrombocytopenic purpura
IUIS	international union of immunological societies
IQR	inter-quartile range
IVIG	intravenous immunoglobulins
JBZ	Jeroen Bosch hospital

yrs	years
LISS	Longitudinal Internet Studies for the Social sciences
LLNR	lower limit of normal range
LOCID	late onset combined immunodeficiency
LRBA	lipopolysaccharide responsive beige-like anchor protein
MHC	major histocompatibility complex
MOOSE	meta-analysis of observational studies in epidemiology
NFKBIA	nuclear factor of kappa light chain gene enhancer in B cells inhibitor alpha
	natural killer
PAD	predominantly (primary) antibody deficiency
PCV	pneumococcal conjugate vaccine
PID	primary immunodeficiency
Pn-C	conjugated pneumococcal
PnPS	pneumococcal polysaccharide
PP	pneumococcal polysaccharide
PVR	pneumococcal vaccination response
RAST	radioallergosorbent test
ROC	receiver operating characteristics
SAS	stichting voor afweerstoornissen
SD	standard deviation
SE	standard error
sIgAdef	selective IgA deficiency
sIgMdef	selective IgM deficiency
SLE	systemic lupus erythematosus
SPAD	specific antibody deficiency
STROBE	strengthening the reporting of observational studies in epidemiology
TAAQOL	TNO-AZL questionnaire for adult's HRQoL (≥16 years of age)
TACI	transmembrane activator and calcium-modulator and cyclophilin ligand
	interactor
TACQOL	TNO-AZL questionnaire for children's HRQoL (6-15 years of age)
TAPQOL	TNO-AZL questionnaire for pre-school children's HRQoL (1-5 years of age)
THI	transient hypogammaglobulinemia of infancy
unPAD	unclassified primary antibody deficiency
VDJ	variable, diversity, joining
XLA	X-linked agammaglobulinemia